PROBING OF INTRACELLULAR RESPONSES TO PULSED ELECTRIC FIELD IN HeLa CELLS USING FLUORESCENCE LIFETIME MICROSCOPY

Kamlesh Awasthi, Nobuhiro Ohta
Department of Applied chemistry and Institute of Molecular Science
National Chiao Tung University
1001, Ta-Hsueh Road, Hsinchu 30010, Taiwan
E-mail: kaawasthi@gmail.com

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Application of external electric fields has been extensively used in material, nono and bio- and medical-sciences. In a field of bio- and medical-sciences, electric fields having duration of micro to milli second and strengths of the orders of kV cm⁻¹, have widely been used to modulate membrane permeabilization. Exposure of living cells to such electric fields causes accumulation of electric charges at the plasma membrane, resulting in the reversible rearrangement of the plasma membrane with the formation of pores. Such alteration with electric fields induces temporary increase in the membrane permeability, which has been commonly used to deliver extracellular compounds, such as proteins, nanoparticles and DNA, into the cells. On the other hands, when the pulsed electric field with a duration shorter than the charging time of plasma membrane is applied, it is expected that the applied electric field penetrates into a cell before the charge dissipation into the plasma membrane. This may allow modulation in subcellular structure, dynamics and function without any physical damage of the plasma membrane.

In the present study, we have constructed an electrode microchamber for applying the ultra-short (10 to 50 ns) pulsed electric field with strength of kV cm⁻¹ to living cells. The fluorescence lifetime microscopy has been used to investigate the effects of external electric field on intracellular dynamics and the function of living cells. In order to examine such electric field effect, nanosecond (ns) pulsed electric field have been applied to living HeLa cells and the responses were monitored by the fluorescence lifetime microscopy of enhanced green fluorescence protein (EGFP) and endogenous natural coenzymes such as nicotinamide adenine dinucleotide (NADH). The autofluorescence chromophores, such as NADH, are related to cell functions, and the measurements of autofluorescence lifetime imaging of NADH in living cells under the pulsed electric fields are expected to provide much deeper information on cellular conditions.

It is found that apoptosis, i.e., a natural process of programed cell death in which cells activate an intracellular death program and kill themselves in a controlled way, is induced quickly by the application of ns pulsed electric field, indicating that application of nanosecond pulsed electric fields is a unique method to induce apoptosis. The fluorescence lifetimes of EGFP and NADH also change after the induction of apoptosis, which is attributed to the changes in the intracellular environments surrounding the chromophores. It is also found that the electric field effect depends on the pulse width as well as on the amplitude and the frequency of the applied electric field.