別紙様式８

研　究　主　論　文　抄　録

論文題目

和文: メダカの胚発生へのパルスパワーの影響

（英文）Pulsed Power Effects on Embryonic Development of Oryzias Latipes

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主論文要旨

This dissertation presented a study of applying the pulsed electric field on medaka fish eggs. Pulse electric field has attracted a great interest in combination with various fields. Especially, the technique of using pulsed electric field in medicine and biology has been recently attracted attention, known as bioelectrics.

Applications of pulsed electric field to human or animal cells have been studied for medical developments, such as cancer treatment, drug delivery, and gene therapy, with concentration on the effects to the cell actively. Depending on the pulse intensity and duration, variety of cell's responses like structure modifications is shown. It has recently been reported that application of a short pulsed electric field can increase the possibility of electric field interaction with subcellular structures, leading to secondary cellular events such as temporal increase in cell membrane permeability and the induction of apoptosis. The short pulses in the nanosecond range at high voltage amplitudes also can result in the small pore formation. This pore formation called as electroporation produces the increase of the permeability of the cells; ions and other water-soluble substances are able to penetrate the cell through these nanopores.

In this dissertation, medaka fish eggs were used for analyzing the biological effects of nanosecond pulsed electric field in-vivo and during embryonic development. Medaka model was chosen for the current in-vivo research because of having the merit such as short generation time, small genome size, and the availability of several useful strains. Fertilized eggs of strain d-rR medaka were used. The ages of the experimental eggs were newly laid, 24h post-fertilization, and 48h post-fertilization. A magnetic pulse compression (MPC) was employed as pulse generator. This

device generates pulses of 0.5-20 kV pulses with 50-300 ns duration.

Chapter 2 introduced the characteristics of a magnetic pulse compression to generate a large power in extremely short time for investigating the effects on experimental eggs after applying nanosecond pulsed electric field. In addition, the real medaka fish egg was introduced.

Chapter 3 described the basic cell modeling of electric field and explained the results which are the generated hatching rate and growth process with effects of pulsed electric field on medaka fish egg. In this experiment, two mediums adjusted with 1 and 15 mS/cm were used. Depending to the different conductivity, growth process and period of the experimental eggs were confirmed.

Chapter 4 described the investigation of electroporation using propidium iodide. Usually, the magnitude of pulsed electric field is able to induce the disruption of the membrane barrier function as pore formation. In order to observe the electroporative uptake kinetics of the embryo cells in medaka fish egg after pulse application, propidium iodide which is a fluorescence marker was used as showing the PI expression under the fluorescence microscope.

In addition to chapter 5, in order to have more understanding and to obtain quantification of an electroporative process of the yolk sphere's membrane or the blastomere of young eggs, fluorescein isothiocyanate marker experiments were performed. In this experiment, after injecting fluorescein isothiocyanate into yolk sphere or blastomere of the experimental egg, the phenomenon that occurs in the egg by pulse application was observed under the fluorescence microscope. Both propidium iodide and fluorescein isothiocyanate are able to give some information about the variation of internal structure by pulse application.

The purpose of this thesis is to expand the area of nanosecond pulsed electric field application so as to use a nanosecond pulsed electric field to modify or control embryonic cell development.