Study on the temperature measure of the gene transfection apparatus

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Electroporation is a physical process that transiently permeabilizes prokaryotic and eukaryotic cell membranes with an electrical pulse, permitting cell uptake of a variety of biological molecules. Because of the advantages of high efficiency, low toxicity popularity, and controllability, it has been applied in the fields such as cell engineering, genetic engineering, and so on. The principles and characteristics of gene transfection apparatus which is made by us will be illustrated in this paper. The temperature influence is considered and the optical fiber sensor in the apparatus is proposed.

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Most known biological effects of externally applied electric fields are based on a field-induced change of the transmembrane voltage[1]. The basic effects of an electric field on a cell can be described by considering the cell to be a conductive body (the cytoplasm) surrounded by a dielectric layer (the surface membrane). When a short electric pulse is applied to this cell, the resulting current causes accumulation of electrical charges at the cell membrane and consequently a voltage across the membrane. If the membrane voltage exceeds a critical value, structural changes in the surface membrane occur with transmembrane pore formation, a process known as electroporation. One of the most widely accepted uses of electrooration technology is the transformation of bacterial, plant, and mammalian cells. It is a quick and highly efficient method applicable to practically all cell types. It has been shown that at subcritical field strengths, proteins can be inserted into the membranes. This has generated much interest, particularly for treatment of AIDS[2]. As we know, the temperature is an important parameter in the electroporation. Both application and mechanistic understanding have received growing attention during the past two decades.

The analytical calculation of the induced transmembrane voltage $\Delta\phi$ caused by a uniform direct electric field across a homogeneous membrane is based on the assumption that the cell is spherical[3]. This postulation is incorrect for many kinds of cells, such as plated cells, cells in tissues, and rod-shaped bacteria; furthermore, the analytical calculations also do not apply in case of a charged membrane surface. For a spherical cell with no surface charge, the position-dependent $\Delta\phi$ is

$$\Delta\phi_{\rm m}(t) = 1.5\alpha E_0 \cos\theta \left[1 - \exp\left(-\frac{t}{\tau}\right)\right], \qquad (1)$$

where E_0 is the strength of the electric field, α is the cell radius, θ is the polar angle measured with respect to the direction of the field, and τ is the time constant of the membrane which reads

$$\tau = \alpha C_{\rm m} \left(1/\sigma_i + 1/2\sigma_{\rm e}\right), \qquad (2)$$

where $C_{\rm m}$ is the membrane capacitance, $\sigma_{\rm e}$ is the external electrolyte conductivity, and σ_i is the cytoplasm conductivity. In Fig. 1, the cell is a sphere with radius α. The external electric field is homogeneous, and E is the absolute value of the electric field strength vector.

The gene pulser apparatus is a pulse generator which uses capacitor discharge to generate electrical pulses for cell electroporation. The key point is the apparatus can produce controlled high voltage and strong current.

The gene pulser apparatus produces controlled exponential pulses with peak amplitudes from 300 to 2500 V. The gene pulser apparatus offers selectable capacitance for energy delivery with longer time constants. Together the gene pulser apparatus and Capacitance Extender can produce pulses of up to 2250 V/cm with time constants from 5 μs to 1 s, depending on sample resistance.

The gene pulser apparatus has been widely used in many fields, but because of electromagnetic interference of currents and voltages in the metallic conductors, the temperature measurement is a difficult problem. Electroporation is a non-thermal effect. Absolutely, the temperature is an important parameter in the experiment. It is not clear how electromagnetic field affects the catalysis mechanism and catalyst. One of the reasons is that the temperature can not be measured in strong electromagnetic field intensity accurately. At the same time, the enzyme is sensitive to the temperature. In order to offer unparalleled efficiency and reproducibility, we will propose a solution.

Basically, there are three possibilities for temperature measurement: 1) temperature measurement using thermocouple devices; 2) a single-point fiber-optic system; 3) a distributed measurement fiber-optic system.

The easiest method (using thermocouple devices) provides information on the oil temperature at the top and bottom of the cooling unit. However, the closer the temperature measurement is carried out to the object, the more accurate the approximation of the measured temperature will be. In addition, measurements based on traditional sensors are not dependable since they are

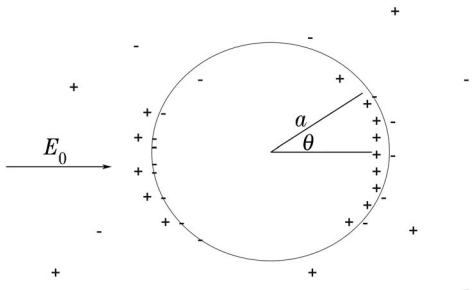

Fig. 1. The cell in the electric field.

characterized at least by uncertainty due to electromagnetic noise. Recently, fiber-optic systems for point measurement and for distributed measurement along the object have been tested for temperature monitoring within large power transformer[4]. The accuracy obtained is sufficient for on-line monitoring: $\pm 1^\circ$ for point sensors, and $\pm 1^\circ$/m for distributed measurement systems.

Worldwide, there is a growing demand for the electroporation monitoring of bacterial, plant, and mammalian cells in order to increase safety as well as efficiency. One of the most promising candidates for advanced practical solutions is the optical fiber Bragg grating (FBG) as an intrinsic sensor for strain, temperature, and other measurands of interest in such monitoring applications. FBG sensors have a number of significant technical advantages compared to competitive electrical transducers. They can be multiplexed, embedded in composite materials, applied in adverse environment, and large-area distributed sensor networks with low signal transmission losses can be realized. Therefore, during the last decade in numerous industrial countries many activities and pilot projects on the development and application of this novel sensor technology have been started. In the following, the principle of temperature sensor will be introduced.

Figure 2 schematically shows an accurate temperature sensor system based on the optical FBG theory. According to the coupled mode equations, we can analytically derive the reflectivity of each FBG. The original wavelength FBG is[5]

$$\lambda_{\mathrm{B}} = 2n_{\mathrm{eff}}\Lambda, \tag{3}$$

where Λ is the grating period and n_{eff} is the fiber refractive index. When the temperature changes with amount ΔT, the original wavelength λ_{B} will be changed as

$$\Delta\lambda_{\mathrm{B}} = \lambda_{\mathrm{B}}\left(\alpha + \gamma\right)\Delta T, \tag{4}$$

where$\alpha = (1/\Lambda)\,(\Delta\Lambda/\Delta T)$ is the thermal expansion coefficient, $\gamma = 1/n_{\mathrm{eff}} = (1/n_{\mathrm{eff}})(\Delta n_{\mathrm{eff}}/\Delta T)$ is thermo-optic coefficient. The temperature can be obtained by measuring changed wavelength because of the linearization between ΔT and $\Delta\lambda_{\mathrm{B}}$.

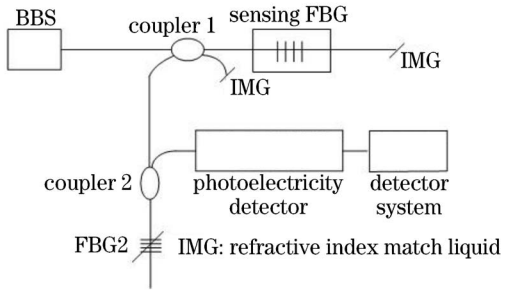

Fig. 2. The configuration of an accurate temperature sensor system.

To sum up, the optical fiber Bragg grating sensor is effective and flexible in practice because it can arranged at any predetermined position along the fiber link. Especially, it can be used in gene pulse transformation experiment.

In summary, the gene pulser transfection apparatus and the optical fiber sensor thermometer comprise a flexible system for electroporation of eukaryotic or prokaryotic cells. Proteins, nucleic acids, and other molecules can be introduced by electroporation into many different types of cells. The microprocessor-controlled gene pulser transfection apparatus and the thermometer offer unparalleled accuracy and reproducibility.

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