THE EFFECT OF PULSED ELECTRICAL FIELDS ON BIOLOGICAL CELLS

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Abstract

Experimental studies on the effect of pulsed electric fields on aquatic organisms and on cancer cells have shown that the stress level of micro-and macro-organisms can be controlled by varying the pulse amplitude and pulse duration. It could also be shown that an increase in frequency, or reduction in pulse duration, respectively, causes the location of the cell damage to change from the outer membrane to the nucleus. The experimental results can be explained by considering the equivalent circuit of biological cells.

Introduction

Biological cells consist of cytoplasm surrounded by a membrane. The cytoplasm is conducting, the membrane, which consist of a lipid bilayer, can be considered a dielectric. The application of electric fields to biological cells in a conducting medium, e.g. water, causes buildup of electrical charge at the cell membrane, and consequently a change in voltage across the membrane. For low electric fields, such that the voltage across the membrane is on the order of ten's of millivolts, voltage induce opening of channels in the membrane causes flux of ions through the membrane, changes the ion concentration close to the cell membrane, and consequently cell stress. This stress lasts on the order of milliseconds, and does not cause cell damage. If the electric field is increased such that the voltage across the cell membrane reaches levels on the order of one volt, the membrane permeability increases to such a level that either the cell needs from seconds to hours to recover (reversible breakdown), or cell death may occur. The mechanism of the membrane breakdown is not well understood. A common hypothesis is that pores are generated in the membrane of sizes which allow the exchange of macromolecules which for high transmembrane voltages do not close anymore [1] .

The effect of electric fields on biological cells, inducing stress, causing reversible or irreversible membrane breakdown, is not just dependent on the magnitude of the field, but also on its duration. Experiments on bacteria showed that reducing the time for which the electrical field was applied required to increase the amplitude of the electric fields in order to obtain a certain effect [2]. However, this increase in field amplitude was found to be such that the electrical energy, which is proportional to the square of the electric field times the pulse duration, decreases with reduced time (at least for pulses longer than 50 μs). Pulsed power technology seems therefore to offer the possibility to optimize methods which rely on the electro-manipulation of biological cells.

The possibility to use pulsed electric field to induce stress or mortality in biological cells has some interesting applications. Pulsed electric fields may be used to kill unwanted cells, e.g. bacteria in liquid food, or cancer cells in human tissue. They might also be used to reduce the activity of cells in a micro-organism for a defined period of time. This electromanipulation prevents certain unwanted activities of a nuisance species without killing the organism. We have concentrated on two applications: use of

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moderate pulsed electric fields to stun aquatic nuisance species, which otherwise would cling to the interior of pipes (e.g. in cooling water systems) and then through uncontrolled growth cause clogging of the pipes (biofouling), and the use of pulsed electric fields for the elimination of cancerous cells.

Electrical Equivalent Circuit of a Biological Cell

A simple electrical equivalent circuit for a biological cell in a suspension, shown schematically in Fig. 1a, which does not take into account the effect of structures inside cells , such as the nucleus, is shown in Fig. 1b. The suspension is modeled by a resistance and capacitance. For pulse duration long compared to the dielectric relaxation time of the suspension, the capacitive component of the impedance can be neglected. For many suspensions and for sea water the dielectric relaxation time is on the order of ns [3]. The cell membrane is modeled as capacitor, the cytoplasm as resistor. The outer membrane contains channels which controlled by the applied voltage allow flow of ions through the membrane, representing a leakage current. The voltage-gated channels are modeled as variable, voltage-dependent resistors [4].

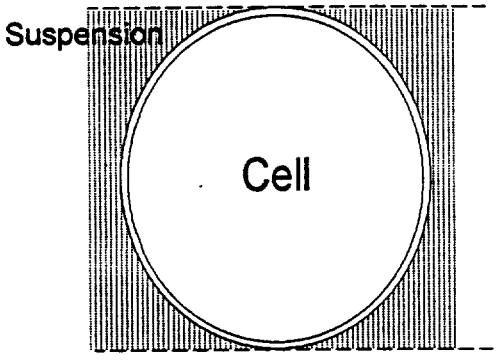

Fig. 1a: Cell in Suspension

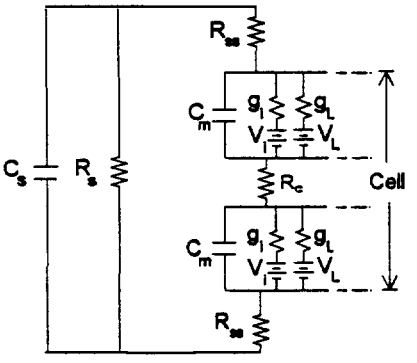

Fig. 1b: Electrical Equivalent Circuit [3]

When a voltage pulse is applied to the cell, charges accumulate at the membrane and the membrane voltage is increased. At a certain voltage level which is on the order of 1 V, reversible breakdown will occur; at even higher voltage levels the cell will suffer irreparable damage. The electric field in the suspension required to generate a voltage, V, across a cell with radius a_{cell} is:

$$E = V/fa_{cell} \qquad [1]$$

where f is a form factor, characteristic for the shape of the cell [5]. The charging time constant of the cell membrane is given as:

$$\tau = (\rho_s/2 + \rho_c)\, Ca_{cell} \qquad [2]$$

with ρ_s being the resistivity of the suspending medium, e.g. water, ρ_c being the resistivity of the cytoplasm, and C the membrane capacitance per unit area [6]. Using typical data for cells we can now calculate the duration of the electric field pulses required to generate 1 V across the membrane. The energy, W, dissipated in the suspension is:

$$W = E^2\tau/\rho_s \qquad [3]$$

The calculated electric field and the corresponding energy density, W, versus the pulse duration is shown in Fig. 2.

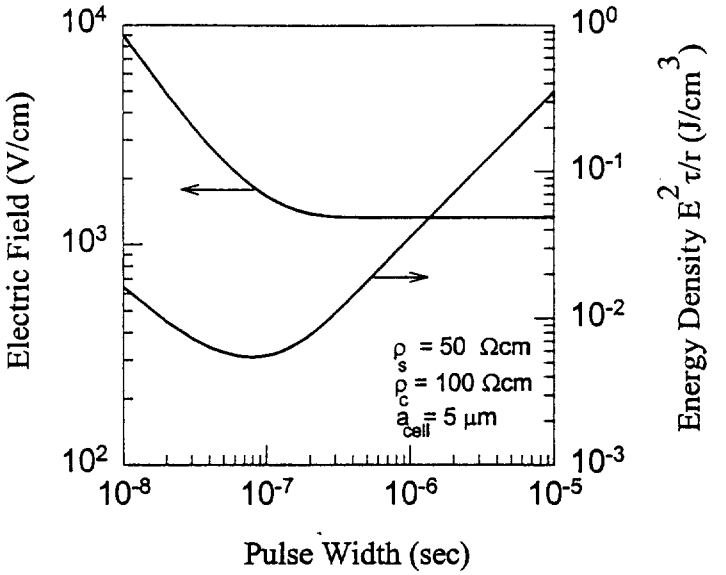

Fig. 2: Electric Field in Suspension Required to Charge the Membrane to 1 Volt, and Corresponding Energy Density versus Pulse Duration

The curves show a minimum at 0.1 μs. This is the pulse duration where stunning or killing of this type of biological cells is most effective. Experimental observation has confirmed the presence of such a minimum [3]. In any application of this method, if it is debacterialization of food and liquids, treatment of cancer, prevention of biofouling, the optimization of the method requires to find this minimum in energy consumption.

Experiments

Stunning of aquatic nuisance species as a method to prevent biofouling

Pulsed electric fields have been shown in previous studies to affect the settling of Zebra mussels [7]. The shortest pulse duration, τ, in the studies reported in [7] was on the order of ms for Zebra mussels, and 10 μs for Daphnia. In order to determine the optimum operation of the pulsed electric field method for biofouling prevention we have studied the effect of pulsed electric fields on aquatic nuisance species over a wide range of pulse duration and electric field parameters, in both laboratory [3] and field experiments [8]. The pulse power systems which have been used in the lab experiments have been described in reference [3]. The field system consists of a 7 Ω Blumlein-type pulse generator with a thyratron as switch. The load consists of a volume of water between two plane electrodes.

In field studies water from the Elizabeth River in Norfolk, VA, an estuary of the Chesapeake Bay, was pumped through a treatment cell, where the voltage pulse was applied, and through an identical control cell without any voltage applied. The resistivity of the tidal water was on the order of 50 Ωcm. After passing through the cells the water flew through 4.5 m long , 1.5 cm inner diameter PVC pipes, and was then discharged into the river. Part of the results of a series of experiments with electric fields varying from 12 kV/cm to 0.75 kV/cm and a pulse duration of 0.77 μs at a repetition rate of 12 Hz, are shown in Fig. 3 [8]. Plotted is the efficiency of the pulsed electric field method, which is defined as one minus the ratio of the number of barnacles found in the treatment tubes to that in the control tubes. For electric fields of > 5 kV/cm the method provides 100% protection against biofouling by barnacles. It decreases with decreasing electric field, but even at 1 kV/cm, the efficiency is still above 90%, that means for 10 barnacles in the control tubes we see only one in the tubes where the treated water flows.

The experimental results obtained in tidal water, and the theoretical studies on the effect of the water resistivity on the power consumption [3], allow us to estimate the cost of biofouling prevention in a fresh water environment. For a 99% efficiency of the method in tidal water with a resistivity of 50 Ωcm, electrical fields of approximately 3 kV/cm are required (Fig. 3). At a pulse duration of 0.77 μs the amount of water treated per unit energy is 26,000 liter/kWh. In other words, the cost of electrical energy is on the order of 10 cents for the treatment of 7,200 gallons of tidal water.

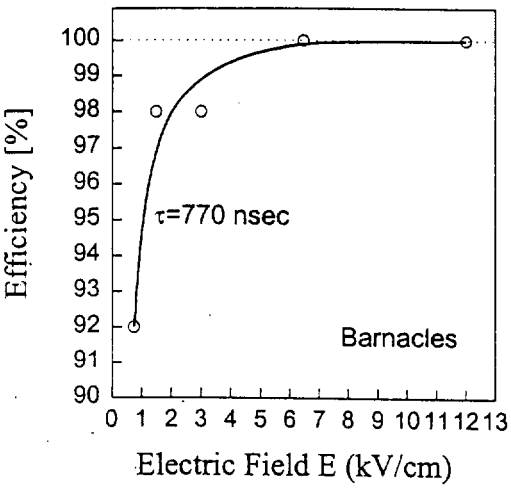

Fig. 3: Efficiency of Pulsed Electric Field Method in Preventing Growth of Barnacles in a Cooling Water System dependent on Electric Field Strength

Equ. 3 seems to indicate that increasing the resistivity of water from 50 Ωcm (tidal water, or sea water) to 5,000 Ωcm (fresh water) causes an increase by a factor of hundred in the amount of water treated per unit energy. However, modeling studies, where the biological cell was described in terms of electrical components, indicate that the absolute gain is smaller, more on the order of ten, and that it requires longer pulses to obtain optimum results [3]. Still, it should be possible to treat approximately 250,000 liter of fresh water with an energy of 1 kWh, or at the cost of 10 cents, and get results comparable to those obtained in tidal water with field of 3 kV/cm applied: a 99% reduction in biofouling.

Study of the effect of pulsed electric field on cancer cells

HL-60 Leukemia cells were used to study the effect of pulsed fields on cancer cells. A typical cell is shown in Fig. 4a. The nucleus is clearly visible. Smaller substructures, nucleoles, are visible in the original, but not in the copy shown in this paper. The substructures can be modeled by treating the membrane surrounding the nucleus as a capacitance and the interior of the nucleus as a resistor, both elements in series to the resistance which describes the cytoplasm in the first, simplified, equivalent circuit (Fig. 4b). Similarly, the nucleoles can be described by a capacitor resistor arrangement in parallel to the nucleus resistance.

Fig. 4a: Leukemia Cell

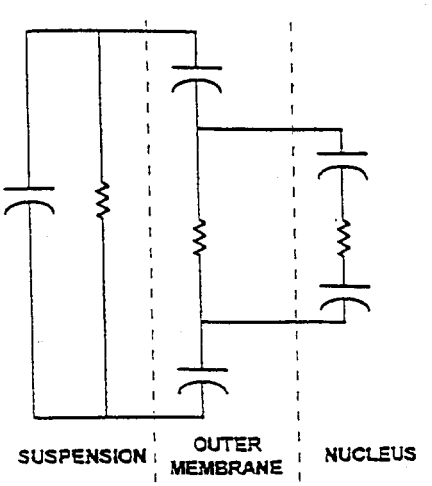

Fig. 4b: Electrical Equivalent Circuit

From basic circuit principles it is clear that low frequency electric fields affect mainly the outer membrane. With increasing frequency, the outer membrane, however, will be effectively shortened out, and the applied voltage will appear across the inner (nucleus) membrane. This behavior is shown in Fig. 5, where the voltage across the outer membrane and that across the nucleus membrane is plotted versus frequency. It shows that at frequencies around 1 MHz, the nucleus membrane is more prone to destruction through overvolting than the outer membrane, or, shorter pulses, with higher frequency components are expected to affect the nucleus of a cell rather than the cell membrane.

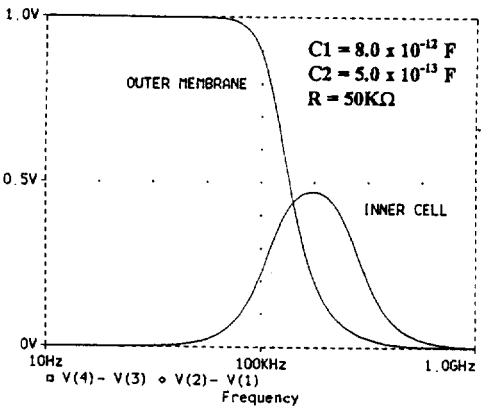

Fig. 5: The Applied Voltage across the Outer and the Nucleus Membrane versus Frequency

Experiments were performed where Leukemia cells in cuvettes with plane parallel electrodes of 1, 2, and 4 mm separation [3], were exposed to 5 μs, 1 μs, and 50 ns long electric pulses. The pulse generator for the 50 ns pulses is described in reference [3], for the microsecond pulses we have used a pulse generator with a MOSFET as switch. Two methods have then been used to evaluate the effect of pulsed electric fields on the human cells:

a) viability tests with Trypan Blue,

Trypan Blue is a viability stain that was added to the solution after the cells were treated with electric fields. It is only introduced into the cell if the outer membrane is damaged, and serves to identify cells where the outer membrane is severely damaged.

b) morphology tests with Wright's stain,

The treated cells and untreated (control) cells are dyed with Wright's stain. This stain shades the different parts of the cell differently, and allows us to see changes in the morphology of the cells.

The results are as expected: for long pulses (5 μs) the outer membrane is predominantly damaged. A photograph of cells exposed to pulses of this duration at an electric field of 1.4 kV/cm (Fig. 6 a) shows the outer membrane damaged, but the nucleus, and particularly the nucleolus (not visible in the copy) intact. Reduction of the pulse duration to 50 ns, but keeping the total energy of the pulse roughly the same (by increasing the electrical field to 13.5 kV/cm) causes damage of the nucleus but not to the outer membrane. This is shown in Fig. 6b. The mortality under this short electric field exposure is generally not instantaneous. But in a follow up measurement of the viability of the cells, it was found that after three days 90 % of the exposed cells had died, whereas in the same time the control group increased in number by a factor of two.

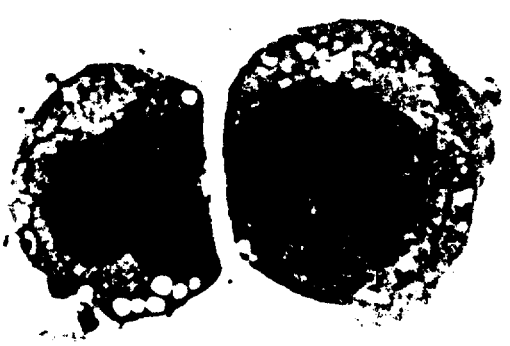

Fig. 6a

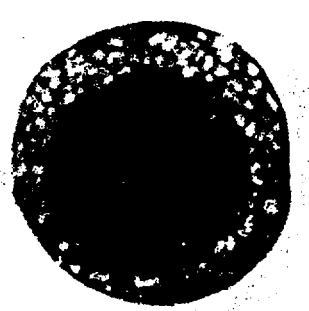

Fig. 6b

Fig. 6: The Effect of Long (5μs) Pulses (6a) and Short (50 ns) Pulses (6b) on Leukemia Cells. The Energy is in both Cases The Same.

Conclusion

Pulsed electric fields, with amplitudes in the kV/cm range and with duration of microsecond and less, have been shown to cause cell stress and, and at high fields, cell mortality. This effect has applications in the prevention of biofouling, and debacterialization of liquids. Variation of the pulse duration allows us to damage parts of the cell selectively: longer pulses affect the outer membrane, shorter pulses the substructures of the cell. It might enable us to target cells with different substructures selectively, an application which could lead to treatment of certain types of cancer.

Acknowledgment

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