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An experimental system for controlled exposure of biological samples to electrostatic discharges

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ABSTRACT

Electrostatic discharges occur naturally as lightning strokes, and artificially in light sources and in materials processing. When an electrostatic discharge interacts with living matter, the basic physical effects can be accompanied by biophysical and biochemical phenomena, including cell excitation, electroporation, and electrofusion. To study these phenomena, we developed an experimental system that provides easy sample insertion and removal, protection from airborne particles, observability during the experiment, accurate discharge origin positioning, discharge delivery into the sample either through an electric arc with adjustable air gap width or through direct contact, and reliable electrical insulation where required. We tested the system by assessing irreversible electroporation of *Escherichia coli* bacteria (15 mm discharge arc, 100 A peak current, 0.1 μs zero-to-peak time, 0.2 μs peak-to-halving time), and gene electrotransfer into CHO cells (7 mm discharge arc, 14 A peak current, 0.5 μs zero-to-peak time, 1.0 μs peak-to-halving time). Exposures to natural lightning stroke can also be studied with this system, as due to radial current dissipation, the conditions achieved by a stroke at a particular distance from its entry are also achieved by an artificial discharge with electric current downscaled in magnitude, but similar in time course, correspondingly closer to its entry.

1. Introduction

Electrostatic discharges have long been known to humans; in nature we encounter them in the form of atmospheric lightning strokes, while artificial electric arcs – the first one generated by Humphry Davy in 1809 [1] – have many fields of application, ranging from light sources (arc lamps, including fluorescent tubes) to a wide span of tools for processing of materials, particularly for welding, heating (arc furnaces) and plasma cutting. Recently, it was reported that nanosecond electric arcs (sparks) applied to living skin lead to a more efficient DNA uptake and expression than the standard approach in which electric pulses are delivered through electrodes in direct contact with the skin [2], and it was also suggested that lightning strokes could have contributed to DNA transfer during evolution [3–5].

The physics of the effects caused by electrostatic discharges interacting with simple materials is well understood. Most technological processes thus exploit the heat dissipated by the electric current of the arc (flowing either through the material or through the air nearby), while emission of light from arc lamps also involves ionization and quantum excitation of the gas through which this current flows. When flowing through a material, the electric current of the arc also induces an electric field in the material, which is the strongest at the current's point of entry, then gradually decreases as the current flow is dispersed over larger cross-sections inside the material, and – provided that its exit is also point-like – increases again to reach another peak at the point of exit.

When an electrostatic discharge interacts with living matter, the basic physical effects – the induced electric field, the temperature increase, and possibly ionization caused by the electric current – are the same as in simpler materials, but they can be accompanied by a range of biophysical and biochemical phenomena. Electric fields as weak as 60 mV/cm (for durations over 100 μs) excite nerve and muscle fibers, while much stronger fields (hundreds of V/cm or more) cause – in all cells, both excitable and non-excitable – a considerable increase of their membrane permeability (electroporation) and/or their merger (electrofusion). If the field is neither too strong nor too long-lasting, electroporation is reversible, while otherwise it becomes irreversible, resulting in cell death. With sufficient power dissipation, exposures to strong electric fields also cause thermal damage to the cell and its molecules (protein denaturation, DNA melting).

The phenomena of electroporation and electrofusion have been known for several decades. Electroporation was thus first reported for an excitable cell plasma membrane (Ranvier node of a myelinated axon) in 1958 [6], for a non-excitable cell plasma membrane (bacterial outer and cytoplasmic membrane) in 1967 [7], for organelle membrane (of a chromaffin granule) in 1972 [8], and for a planar lipid bilayer (oxidized cholesterol/n-decane) in 1979 [9]. Electrofusion was first demonstrated for animal cells (both anucleate and nucleated) in 1980, and for plant protoplasts and lipid vesicles in 1981 [10–13].

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Since their discovery, electroporation and electrofusion have been extensively investigated and have to date found multiple applications in medicine and biotechnology. In medicine, reversible electroporation is used for delivery of chemotherapeutics in cancer treatment [14] and of DNA in gene therapy [15], irreversible electroporation is a promising technique of tissue ablation [16], while electrofusion holds some promise in preparation of monoclonal antibodies for both diagnostics and therapeutics [17]. In biotechnology, irreversible electroporation is an efficient technique for extraction of biomolecules from cells and tissues [18,19], and is also useful – either accompanied by thermal effects or not – for inactivation or/and destruction of microorganisms [20].

Although some unknowns still remain, the physical properties of electroporation and electrofusion are by now generally well understood on the cellular and membrane level, while the recent molecular dynamics simulations are also improving our understanding of both phenomena on the molecular and atomic level [21–25]. Nevertheless, the mechanisms of electroporation-mediated transport, particularly of macromolecules such as DNA, are still a subject of vigorous investigation, as they likely involve several concurrent phenomena generated by the electric field pulses either directly (electroporation) or indirectly — by the resulting pressure waves (sonoporation) and/or thermal effects (thermoporation) [26,27].

Electroporation is, according to both theoretical considerations [28,29] and molecular dynamics simulations [22,23], an electric field-induced formation of aqueous pores in lipid parts of biological membranes. In this process, the water molecules penetrate into the lipid bilayer and interact there with adjacent membrane lipids that consequently reorient with their polar heads towards these water molecules, thus forming a polar pore wall. These pores render the membrane locally permeable to both ions and molecules. Electroporation occurs in the lipid bilayer of the membranes of all prokaryotic and eukaryotic cells, with the pores in the plasma membrane providing a pathway for transport of a wide range of molecules, including DNA, into [26] and out of the cell [7]. Pore formation is governed by electrochemistry and statistical thermodynamics [28,29] and due to the latter it is not strictly a threshold event, in the sense that the pores would only form in electric fields exceeding a certain level, but transport across the electroporated membrane is strongly correlated with transmembrane voltage induced by the electric field [30], which is in turn proportional to the strength of this field [30].

Electrofusion of two cells can occur both if they are in direct contact during the exposure to the electric pulses, or if they are brought into such contact within a sufficiently short time (seconds or even minutes) after the exposure [31,32]. Experiments also show that in electrofusion of two lipid bilayers, the monolayers in direct contact often fuse first, while the other two monolayers still appear intact [33]. This suggests that electrofusion proceeds in the same three stages broadly recognized in the physiological fusion of two cells in direct contact: first, their membranes' external monolayers, at least one of which is locally destabilized, fuse within the area containing the instability, forming a stalk; second, the fused monolayers move apart radially, forming a disk-shaped diaphragm and bringing the internal monolayers into contact; and finally, the rupture of the diaphragm creates a pore connecting the cytoplasms of the two cells, thus completing the fusion [34]. Physiological fusion and electrofusion then differ mainly in the trigger of local destabilization of the exterior monolayer that initiates the fusion — various fusogenic membrane proteins in the former case [35], and electric pulses in the latter [36].

In medical and biotechnological applications of electroporation and electrofusion, as well as in basic research of these phenomena and the accompanying thermal effects, the required electric fields are generated by a suitable voltage source and delivered through electrodes in direct contact with the sample. Furthermore, while the early voltage sources used for electroporation and electrofusion were based on a capacitor discharge and as such delivered exponentially decaying pulses, the modern sources largely deliver rectangular pulses, with the voltage turned on stepwise, sustained at a constant level for a preset duration of the pulse, and then turned off stepwise. Such a description is a slight idealization, but the rise- and falltimes of the commercially available rectangular pulse generators for electroporation are now well below a microsecond, and the variability of the pulse amplitude is in general within a few percent of the preset value. In this manner, the electrical parameters used for electroporation and/or electrofusion are well controlled, making the basic studies reproducible and the resulting applications reliable.

Electric fields with amplitudes and durations adequate for electroporation and electrofusion can also occur in natural environments when these are hit by a lightning stroke [4,5]. But unlike with the laboratory studies and applications of electroporation and electrofusion described above, a stroke proceeds through a highly conductive channel (electric arc) created by electrical breakdown of the air separating the cloud and the ground, and the time course of the electric current and the electric field induced by it as it flows through the ground are neither rectangular nor purely exponentially decaying. Furthermore, in the ground the current does not flow towards a well-defined electrode, but dissipates downward and outward from its point of entry, and consequently the amplitude of the electric field it induces decreases rapidly with increasing distance from this point.

To adequately study lightning-induced electroporation, electrofusion, and the accompanying physical, biophysical and biochemical effects, the standard equipment used in the studies and applications of electroporation and electrofusion would thus have to be adapted to reflect the abovementioned distinctions — the delivery of the electric current to the sample through an electric arc formed in the air, the current's roughly radial dissipation in the sample, and preferably also its specific time course.

In this article, we describe the design, construction and testing of a scalable modular exposure system built along the above-mentioned guidelines, allowing to expose samples of biological cells and tissues to an electrostatic discharge with an adjustable peak current (up to several hundred amperes) in a controlled environment (with a precisely defined arc length, and with monitored time course of the current flowing through the sample). This provides a reproducible emulation of a downscaled lightning stroke. The exposure system allows the researchers to incorporate the electrostatic discharge generator and the ground-simulating electrode(s) of their choice, to quickly adjust the arc length, and due to its modular nature the system also allows for quick assembly, disassembly, and thorough cleaning.

2. Materials and methods

2.1. System development

2.1.1. Computer modeling

The components of the exposure system and the construction of the system as a whole were first modeled computationally in SolidWorks 2013 (Dassault Systèmes SolidWorks Corporation, USA), which was used both for mechanical design and for material selection, with the latter also comprising a basic numerical assessment of the mechanical strength of the materials. For more advanced numerical calculations of lightning stroke simulations and electric field distribution within the exposed sample, we used COMSOL MultiPhysics 4.2 (Comsol, Stockholm, Sweden).

2.1.2. Materials

The main construction material was polyethylene plastic (PE 500 Natur, Simona AG, Germany), as it is a good electrical insulator, widely available, affordable, and easy to clean. To allow for visual monitoring of the sample during the experiment, transparent parts of the system were constructed from plexi-glass (PLEXIGLAS XT Tube Clear 0A070GT, Evonik Industries, Germany), which is also a good insulator and easy to clean. For the electrodes, we tested both copper and stainless steel,