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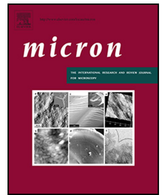

Effects of nanosecond pulse electric fields on cellular elasticity

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ABSTRACT

We investigated the effects of a single 60 nanosecond pulsed electric field (nsPEF) of low (15 kV/cm) and high (60 kV/cm) field strengths on cellular morphology and membrane elasticity in Jurkat cells using fluorescent microscopy and atomic force microscopy (AFM). We performed force displacement measurements on cells using AFM and calculated the Young's modulus for membrane elasticity. Differential effects were observed depending upon pulsing conditions. We found that a single nsPEF of low field strength did not induce any apparent cytoskeletal breakdown and had minor morphological changes. Interestingly, force measurements and calculation of Young's modulus showed a significant decrease in membrane elasticity. A single nsPEF of high field strength induced stark morphological changes due to disruption of the actin cytoskeleton and a marked decrease in elasticity likely caused by irreversible membrane damage. We suggest that the cellular morphology is mainly dependent on stabilization by the actin cytoskeleton, while the elasticity changes are partially dependent on the cytoskeletal integrity.

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1. Introduction

The application of electric fields across a biological membrane, causing lipid rearrangements called pores to form, is referred to as electroporation (EP). Depending on the electric field strength, pulse number and duration, the membrane effects can be temporary and will not induce cell death (Neumann and Rosenheck, 1972; Schoenbach et al., 2001). Conventional EP typically consists of pulses with duration greater than 100 μs and sub-kV/cm voltages resulting in the formation of large heterogeneous pores in the plasma membrane (Gabriel and Teissie, 1999; Gowrishankar and Weaver, 2006; Tekle et al., 1990). Cortical actin destabilization was shown not to be related to electropermeabilization of the cell membrane under long pulse durations (Chopinet et al., 2013, 2014).

Nanosecond pulsed electric fields (nsPEFs) are characterized as high voltage (greater than 10 kV/cm), short duration (sub-μs) electrical pulses capable of inducing cellular effects different from conventional EP. NsPEF-related effects include the creation of dense, homogenous nanopores in the cell plasma membrane and pore formation in intracellular membranes, such as organelles and the nuclear envelope (Schoenbach et al., 2001; Gowrishankar et al., 2006; Pakhomov et al., 2009). NsPEFs permeabilize the cell membrane without the uptake of propidium iodide (Pakhomov et al., 2007a,b), unlike conventional EP, and is associated with an increase of intracellular calcium from both intracellular stores and extracellular sources (White et al., 2004; Semenov et al., 2013). The formation of nanopores and penetration into intracellular structures is attributed to pulse durations shorter than the charging time of the cell membrane (Schoenbach et al., 2001; Pakhomov et al., 2007a,b). The permeabilization of external and internal membranes is thought to be the main mechanism of cell death. Previously, we observed that nsPEFs are able to induce chromosome and telomere damage and actin cytoskeleton disruption which was found to also contribute to cell death (Stacey et al., 2003, 2011).

Many investigations have studied the nature of pore formation in cells subjected to nsPEFs while the morphological changes and overall membrane elasticity remain relatively unknown. In this study, we utilized atomic force microscopy (AFM) and fluorescent microscopy to determine the changes in cellular morphology and elasticity.

2. Methods

2.1. Cell culture

Jurkat cells are an immortalized human T-lymphocyte cell line which grows in suspension. Jurkat clone E6-1 (ATCC, Manassas,

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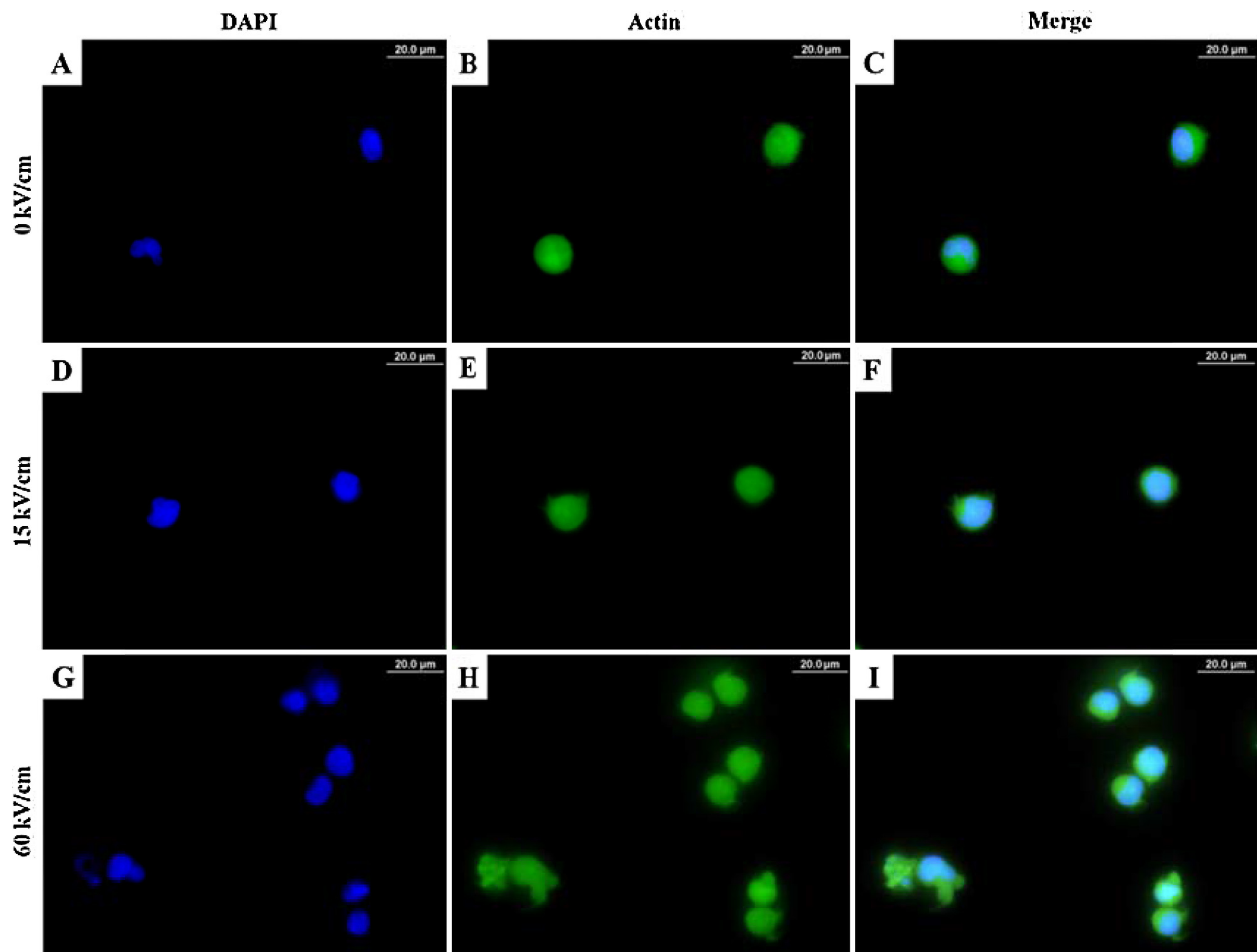

Fig. 1. Actin analysis of cells exposed to nanosecond pulsed electric fields (nsPEFs) using fluorescent microscopy. DAPI (blue) staining of nucleic acids, Phalloidin (green) staining of F-actin, and merged images of Jurkat cells exposed to a single 60 ns pulse of (A–C) 0 kV/cm, (D–F) 15 kV/cm and (G–I) 60 kV/cm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

VA, USA) were cultured in RPMI 1640 medium (Atlanta Biologicals, Norcross, GA, USA) supplemented with 10% fetal bovine serum (Atlanta Biologicals), 2 mM L-glutamine (Gibco/Invitrogen, Waltham, MA, USA), 50 IU/ml penicillin (Gibco/Invitrogen), and 50 mg/ml streptomycin (Gibco/Invitrogen) at 37 °C with 5% CO_2 in air. Cells were maintained in T75 flasks (Thermo Fisher Scientific, Waltham, MA, USA) and fresh medium was added every 2–3 days. Subculturing was performed when cells reached a high density (~3×10^6 cells/ml) and the suspension was centrifuged at $200 \times g$ for 5 min and cells resuspended at 1×10^5.

2.2. Cell electroporation and preparation

For electroporation experiments, cuvettes with 1 mm gaps (BioSmith, San Clemente, CA, USA) were used which have a working volume of 150 µL. The cell suspension was counted and harvested by centrifugation at $200 \times g$ for 5 min, and then resuspended in fresh medium at a concentration of 1×10^6 cells per 150 µL.

The cells were placed in the cuvettes and exposed to a single pulse of 60 ns with pulse strengths of 0, 15, or 60 kV/cm. The pulses were generated using a Blumlein pulse generator as previously described (Schoenbach et al., 2001). After exposure, cells were immediately fixed with 4% paraformaldehyde then transferred to poly-L-lysine coated coverslips (Sigma-–Aldrich, St. Louis, MO, USA). After fixation, cells were washed three times in PBS for 5 min each and then imaged using atomic force microscopy, or the cytoskeleton stained for fluorescent microscopy.

2.3. Cytoskeletal staining

For the cytoskeletal staining, following fixation and washing, the cells were incubated in Oregon Green 488 phalloidin (Gibco/Invitrogen) for 20 min to stain F-actin and then washed three times in PBS + 1% Tween-20 (PBS-T) for 5 min each. The nuclei were counterstained using 1 µg/ml 4′,6-diamidino-2-phenylindole (DAPI; Sigma) in PBS-T for 5 min followed by three PBS-T washes for 5 min each. After a brief rinse in PBS, the coverslips were mounted on slides using VECTASHIELD antifade mounting medium (Vector Labs, Burlingame, CA, USA). Electronic fluorescent images were captured using an Olympus DP70 CCD camera through an Olympus BX51 microscope (Olympus America Inc., Center Valley, PA, USA).

2.4. Atomic force microscopy

The height, phase, and NSOM images of the Jurkat cells were obtained by using a Multiview-4000 multi-probe atomic force microscope (Nanonics Imaging, Jerusalem, ISR) as previously described (Stacey et al., 2013). In brief, the coverslips with fixed cells were loaded onto the AFM stage and images with a resolution of 256×256 pixels were obtained using NWS (Nanonics) and WSxM 5.0 (Nanotech Electronica, Madrid, ESP) software with

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