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Tutorial

Energy dissipation mapping of cancer cells

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ABSTRACT

The purpose of this study is to map the energy dissipation of Jurkat cells using a single 60 nanosecond pulse electric field (NsPEF), primarily through atomic force microscopy (AFM). The phase shift is generated by the sample elements that do not have a heterogeneous surface. Monitoring and manipulating the phase shift is a powerful way for determining the dissipated energy and plotting the topography. The dissipated energy is a relative value, so the silica wafer and cover slip are given a set reference while the transmission of energy between the tip of the cantilever and cell surfaces is measured. The most important finding is that the magnitude and the number of variations in the dissipated energy change with the strength of NsPEF applied. Utilizing a single low field strength NsPEF (15 kV/cm), minor changes in dissipated energy were found. The application of a single high field strength NsPEF (60 kV/cm) to Jurkat cells resulted in a higher dissipated energy change versus that of in the low field strength condition. Thus, the dissipated energy from the Jurkat cells changes with the strength of NsPEF. By analyzing the forces via investigation in the tapping mode of the AFM, the stabilization of the cytoskeleton and membrane of the cell are related to the strength of NsPEF applied. Furthermore, the strength of NsPEF indicates a meaningful relationship to the survival of the Jurkat cells.

1. Introduction

In 1995, Virgil Elings formulated a method of plotting images using phase shift variation in which that the tapping mode of the AFM can be used to identify the composition change of a heterogeneous surface and to conduct phase imaging via AFM. The topography based on the tapping mode of AFM is obtained by plotting the phase shift of cantilever oscillation. It is indicated that the difference in the phase shift associated with both conservative and non-conservative force found on heterogeneous surfaces can be explained with just a change in the dissipated energy. The phase shift is variable with dissimilar materials. Therefore, a proper measurement requires a reference system (calibration) and its value is calculated according to the sample employed. Topography concerns information pertaining to surface qualities and qualities. However, a phase imaging AFM can obtain inner data, relating to cellular structure, composition, and height variation. The process of the contrast is not well known. The use of AFM phase imaging is a method that will greatly affect nanoscale analysis of samples. Phase imaging AFMs can be used for a wide variety of materials, with a wide range of measurement allowable. Examples of such materials are polymeric materials, cells, proteins, and membranes (Leclère et al., 1996; Brandsch et al., 1997; Magonov et al., 1997; Bar et al., 1998; Krausch and Magerle, 2002; Jeusette et al., 2007)

The use of nanosecond pulsed electric fields (NsPEF) on cells have long been known as a useful tool for inducing electroporation (EP) in the cell membrane. Electroporation can be used to cause temporary and non-fatal effects on the cells, depending on strength of the electric field, number of pulses, and duration of each pulse applied (Neumann and Rosenheck, 1972; Schoenbach et al., 2001). To observe the structure of large heterogeneous pores in the plasma membrane while maintaining cell stability, the pulse durations are not run for more than 100 μs, and a sub-kV/cm voltage is used (Tekle et al., 1990; Gabriel and Teissie 1999; Gowrishankar and Weaver 2006).

Compared to classical EP, the application of NsPEF to cells is characterized by a shorter pulse duration (nanoseconds) than the cell membrane charge time and a higher voltage (greater than 10 kV/cm). One effect of NsPEF is to generate nanopores in the plasma membrane and intracellular membranes (Schoenbach et al., 2007). However, this can result in the death of the cell due to complications from the collapse of the cytoskeleton, damage to intracellular membranes, and externalization of needed proteins (Jeusette et al., 2007; Krausch and Magerle 2007; Neumann and Rosenheck, 1972; Schoenbach et al.,

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2001; Gabriel and Tessie 1999; Gowrishankar and Weaver 2006; Tekle et al., 1990; Binnig et al., 1986). Stacey et al., 2003 investigated at cell survival of Jurkats following exposure to multiple pulses (0, 1, 5, and 10 pulses) at 60 ns 60 kV/cm where cell survival decreased with increasing pulse number.

Electrical interactions measured using AFM provide many useful parameter values for surface charge density, pH, Debye Length, and electrolyte concentration (Binnig et al., 1986; Ducker et al., 1992; Ducker et al., 1994; Butt et al., 2005). By scanning between the silicon nitride surface charge and the cell membrane, we can generate image contrasts. The ability to obtain these results highlights the importance of AFM from an electrostatics point of view.

In the previous papers, NsPEF was induced to distort the membrane of the cells. Using the fluorescent microscopy, variation of cell membrane and cytoskeleton could be observed. DAPI and Phalloidin allowed us to image the cell's form, specifically height and 3D representation. The application of NsPEF at 0 kV/cm intensity indicates that the external appearances of Jurkat cells were rounded. In 15 kV/cm situation, the cell morphology was changed, but the appearance was similar to the case where no electric field was applied. The application of the strength of 60 kV/cm NsPEF had a significant effect on the cytoskeleton of cell. The high strength of NsPEF caused the collapse of the cell morphology and actin cytoskeleton.

2. Young's modulus

In our previous work, we measured Young's modulus using force indentation measurements through our AFM's force spectroscopy mode. Prior to experimentation, it was calibrated based on the parameters from a PDMS standard. The Young's modulus of the PDMS standard was obtained by calculating the force-indentation measurement, which was 550.33 (± 15.70) kPa, and the value was almost the same as the previously calculated value of 549 kPa (Armani et al., 1999). In the elasticity measurement experiment, when the Jurkat cells were exposed to 15 kV/cm NsPEF, the Young's modulus was reduced by about 53%, as compared to the use of 0 kV/cm NsPEF. This result suggested that another factor, such as cell membrane porosity rather than cytoskeleton, had a greater effect on the Young's coefficient reduction. This indicates that the low field strength NsPEF has a significant effect on the cell membrane, consistent with the low field strength experienced in long pulse condition, but not on the actin cytoskeleton (Chopinet et al., 2013; Louise et al., 2014). Its elasticity is reduced and the effect of actin is not related to each other. In contrast, Young's modulus was observed to decrease by 85% in the 60 kV/cm NsPEF. Observing changes in the cytoskeleton indicated that the induced network changes of cytoskeleton affects shape, rigidity, and elasticity of the cells, and that the inverse relationship between the electric field and Young's modulus shows that NsPEF decreases cell membrane stiffness by collapsing the cytoskeleton. It is suspected that the destruction of actin filaments and eventually depolarization occurred; destroying the cell cortex (Berghöfer et al., 2009), and lipid rearrangement in the presence of high field strength pulse strongly affect a cell's morphology and elasticity. We chose Jurkat cells because they are useful for studying susceptibility to cancer treatments, such as radiation and chemotherapy. Additionally, they are spherical, allowing us to better observe changes in the membrane.

2.1. Surface charge density

In our previous work, the exposure of Jurkat cells to 0 kV was set as a control for biochemical measurements. Upon application, the surface charge density of the cells decreased by 81%, with the application of a low NsPEF (15 kV/cm) strength. When the cells were exposed to a high field strength NsPEF (60 kV/cm), there was a larger change in that there was a 125% charge density reduction. Based on the experimental data for each situation, it can be observed that the separation distance decreases and the magnitude of the force increases due to the formation of nanopores and the cellular changes that their formation brings forth. In general, the decline of the effect on the cell membrane is induced as the duration of the pulse decreases because the cell membrane charging time is longer than the pulse. Even after NsPEF, the cell membrane's effects have been described involving effects that are not caused by heat, nanopore formation, and externalization of the protein phosphatidylserine (Vernier et al., 2004, Pakhomov et al., 2009, Beebe 2015) It has been indicated that the protein structure is precisely influenced by NsPEF through the inactivation of the catalytic activity of the cAMP-dependent protein kinase whose function is highly dependent on structure (Beebe et al., 2004, Pakhomov et al., 2009, Beebe 2015). Depending on the orientation of the cells along the electric field, the plasma membrane voltage was led to change in Jurkat cells by NsPEF (Frey et al., 2006), especially when the cell was oriented toward the cathode, generating a greater difference.

In this study, we analyzed the changes of dissipated energy from Jurkat cells in terms of their mechanical and chemical properties. From observation through AFM after exposure of the cells to NsPEF, we demonstrated the correlation of the above changes with varying pulse conditions of NsPEFs.

3. Methods

3.1. Atomic force microscopy

Information about height, phase, and Near Field Scanning Optical Microscopy (NSOM) images of Jurkat cells can be obtained utilizing a Multiview-200 multiple probe AFM (Nanonics Imaging). Interfacing the coverslip with fixed cells with NWS (Nanonics) and WSxM 5.0 (Nanotech Electronica) software with a 20 nm parabolic quartz tip in tapping mode can produce images with a resolution of 256 × 256 pixels. The spring constant of the cantilever is 2600 μN/μm. We employed image calibration using a standard silicon grid and profilometer. Silicon and polydimethylsiloxane (PDMS) were used to examine force measurements. The cells were fixed for Atomic Force Microscopy with 4% paraformaldehyde and placed on cover-slips coated with poly-L-lysine. The cells were subjected to 3 PBS washes of 5 min each before AFM imaging.

3.2. Cell culture and electroporation

Cell culturing, maintenance, and electroporation methods can be found in our prior investigation (Dutta et al., 2015). With RPMI 1620 media via Atlanta Biologicals, Jurkat E6-1 clones from ATCC were cultured at 12°C and 5% CO_2 in a humidified incubator as before. For review, cells were exposed to single pulses of 60 ns with pulse strengths of 0, 15, or 60 via a Blumlein pulse generator described in Schoenbach et al. (Schoenbach et al., 2001). The cells were sub-cultured when they reached a density of 1 × 10^6 cells per 150 μl and were centrifuged at 200 x g for 5 min before being re-suspended and placed in curvettes with 1 mm gaps for pulsing.

3.3. Phase image atomic AFM

Point-mass models were used to analytically express energy transfer from the tip to the surface (García 2011). To preserve the cantilever in a vibrating state with hydrodynamic damping and tip surface dissipation processes, the external excitation energy per cycle, $F_0 \cos \omega t$ (E_{exc}), should equal the per-cycle energy, which is dissipated by the tip-surface forces (E_{dis}) and hydrodynamic damping in the medium (E_{med}), expressed as follows.

$$E_{dis} = E_{exc} - E_{med} \quad (1)$$

The above parameters are represented as (García 2011),