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Assessing the Nano-Dynamics of the Cell Surface

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Abstract It is important to know the mechanism of cell membrane fluctuation because it can be readout for the nanomechanical interaction between cytoskeleton and plasma membrane. Traditional techniques, however, have drawbacks such as probe contact with the cell surface, complicate analysis, and limit spatial and temporal resolution. In this study, we developed a new system for non-contact measurement of nano-scale localized-cell surface dynamics using modified-scanning ion-conductance microscopy. With 2 nm resolution, we determined that endothelial cells have local membrane fluctuations of ~20 nm, actin depolymerization causes increase in fluctuation amplitude, and ATP depletion abolishes all membrane fluctuations.

Keywords: Membrane Fluctuation, Scanning Ion Conductance Microscopy, Micropipette, Actin Depolimerization, Cell Surface

1. Introduction

Thermally [1-5] and actively [6-7] driven cell membrane fluctuations are known to be readouts for the nanomechanical interaction between the cortical cytoskeleton and the plasma membrane and the membrane fluctuation is also strongly determined by the mechanical properties of the cell cytoskeleton and cortical actin filaments [8-12]. It is important to know the mechanism of cell membrane fluctuation because it may play a role in lipid-protein interaction. Some studies have reported that ATP plays an important role in membrane fluctuation. For example, in red blood cells, membrane fluctuations increase as the amount of ATP increase [13,14]. These membrane fluctuations have been revealed by using atomic force microscopy [15], light scattering [14] and optical trapping techniques [16], but the measurement and interpretation of cell membrane fluctuations by these traditional techniques are quite complex.

Scanning ion conductance microscopy(SICM) consists of micropipette probe which filled with electrolyte [17]. As the tip of the micropipette approaches the sample, the ion current decreases since the gap is reduced. By monitoring the ion current, the distance between pipette tip and sample keep constant by applying corresponding voltages to the Z-piezo drive during the scanning procedure. Therefore, the path of the tip follows the contours of the sample surface.

In this study, we developed a new system for non-contact measurement of nano-scale localized-cell surface dynamics using modified- scanning ion-conductance microscopy [18-20]. To position micropipette probe near to the cell surface, we used the ion current like the SICM. However, instead of using scanning mode after the probe positioning, we introduced a new mode which allowed measuring the local membrane fluctuations by monitoring the ion current fluctuations or

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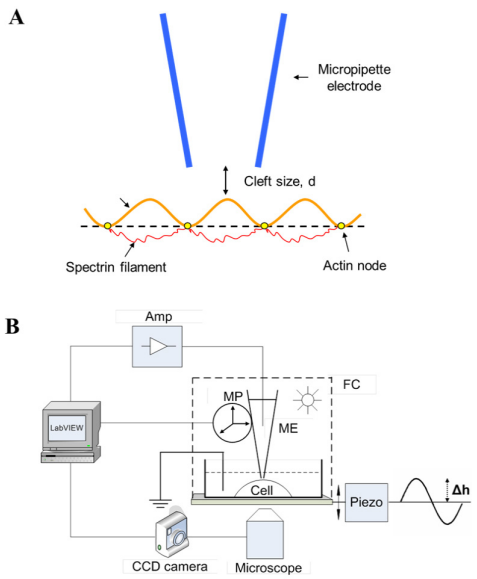

Fig. 1 A: Positioning of electrode near the cell surface. The distance between pipette tip and cell membrane is cleft size and resistance between them is cleft resistance. B: Experimental setup. Delta h is the sine wave amplitude of stage movement. Amp: patch clamp amplifier, ME: micropipette electrode, FC: Faraday cage, MP: micromanipulator, piezo: piezo stage

resistance fluctuations while pipette was stationary near the surface. The Fig. 1A shows the local membrane curvature and one example of cortical filament; spectrin filament [1, 21]. The bilayer is attached to the cytoskeleton through specialized membrane proteins. We defined the distance between pipette tip and cell membrane as cleft size and resistance between them as cleft resistance [17,19-20].

2. Methods

2.1 Cell Culture

Bovine aortic endothelial cells(BAECs) were cultured onto a one-well, chambered #1 coverglass in Dulbecco's modified eagle medium (DMEM) supplemented with 2 mM L-glutamine, 50 units/ml penicillin, 50 mg/ml streptomycin, 1 mM sodium pytruvate, and 10% fetal calf serum (FCS) and maintained at 37°C in a gas mixture of 95% air and 5% CO_2.

2.2 Experimental Setup

The system was comprised of nano-electrode mounted on a motorized micromanipulator and a piezo stage mounted on a microscope. A LabVIEW-based software program coordinated pipette position, piezo stage position, cell imaging, electrical monitoring, and data acquisition. Brief protocol is that we first positioned pipette onto target cell using micromanipulator then bring up the piezo stage toward the pipette tip while monitoring resistance. This positioning stopped automatically when cleft resistance reached a certain value. At this point, holding both pipette and stage stationary, we measured resistance fluctuations.

2.3 Objectives

Four different experiments were performed. First, the system resolution was tested by using 2-10 Hz sinusoidal piezo stage motion with amplitudes ranging from 2 nm to 100 nm. Second, to detect membrane fluctuations, we measured the cleft resistance fluctuations by holding the pipette stationary near cell and reading time traces of cleft resistances. Then, we converted these resistance fluctuations to distance values and get the fluctuation amplitude and frequency spectra by fast Fourier transform. Third, to determine role of actin in membrane fluctuations, we treated cells with 2 μM of actin depolymerizing drug, cytochalasin D, then accessed membrane fluctuations. Finally, to determine role of ATP in membrane fluctuations, we treated cells with ATP depletion drug which was consist of 25 nM antimycin A (AMA) plus 2 mg/ml 2-deoxy-D-glucose, then accessed membrane fluctuations.

3. Results

3.1 Finite Element Model (FEM) Simulation Results

To position pipette non-contractively near the cell and to convert the measured resistance fluctuations to amplitude of membrane fluctuations, we did finite element model simulation for the pipette position with different cleft sizes. As shown in Fig. 2A, most of voltage dropped near the pipette tip. From the simulation, we got the quantitative relationship between cleft resistance and cleft size. Fig. 2B shows both experiment and simulation result during pipette positioning. From at certain distance, cleft resistance increase as cleft size decrease, and simulation result fits well to the experimental one. This graph was used to estimate the distance between cell surface and tip and also used later to convert resistance to distance.

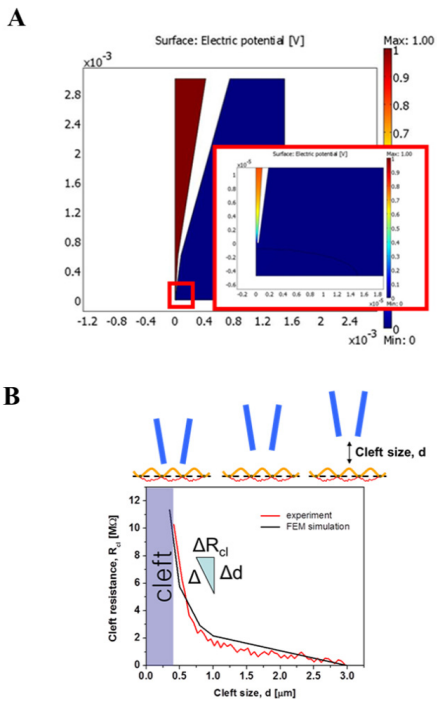

Fig. 2 A: 2D-axial symmetry simulation model and B: Quantitative relationship between R_{cl} and cleft size. This results were used to estimate the distance between cell surface and tip and also used later to convert resistance to distance. Parameters for simulation are shown in the Table 1.

Tabel 1 The dimensions and electrical parameters used for finite element analysis

Conductance of medium	:	0.02 S/m
Conductance of membrane	:	4.7 x 10^{-7} S/m
Cell height	:	4 μm
Cell radius	:	15 μm
Pipette tip Inner radius	:	100 nm
Pipette tip outer radius	:	200 nm
Number of elements	:	~ 680000
Simulated electrode resistance	:	~ 60 MΩ

3.2 Test System Resolution

Frist, we test the system resolution. To do that, we applied sinusoidal waveform into piezo stage with 2 and 5 nm of amplitude. △h is the sine wave amplitude of stage movement. Fig. 3A shows the good match between stage movement and cleft resistance trace. From Fig. 3B, we found that 2 nm movement is detectable with our system.

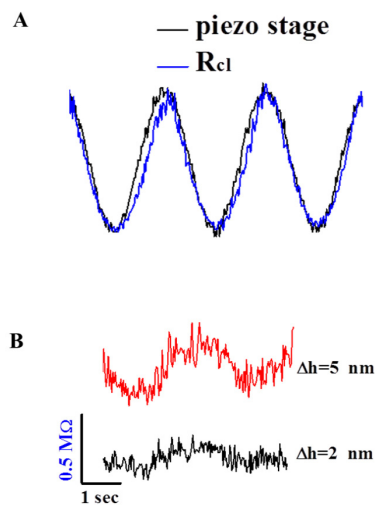

Fig. 3 Sinusoidal waveform was applied into piezo stage to test system resolution. A: good match between stage movement and cleft resistance trace. B: 2 nm movement is detectable with our system.

3.3 Endothelial Cells Exhibit Membrane Fluctuations of ~20 nm at Characteristic Frequencies

With < 2 nm resolution, we measured cell membrane fluctuations. For control experiments, we measured resistance fluctuations onto glass surface without cell which is the system noise. Fig. 4 shows one representative membrane fluctuation. Near at 1300 Hz about 10 nm membrane fluctuation was detected which is not shown in control. We selected this dominant amplitude with 99.9% confidence and averaged those selected at each frequency. From the result, we found that endothelial cells exhibit membrane fluctuations of up to 20 nm at characteristic frequencies(Fig. 5A).

3.4 Actin Depolymerization Causes Increase in Fluctuation Amplitude at All Frequencies

Using normal cell membrane fluctuations as control, we tested the role of actin in membrane fluctuations. Treated cells with actin depolimerizing drug show high membrane amplitude than normal cell. This may be due to the actin depolimerization soften the cells.

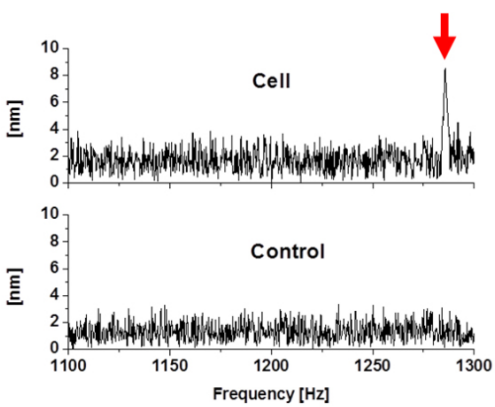

Fig. 4 Determine cell membrane fluctuations and their amplitude and frequency spectra. For control, resistance fluctuations onto glass surface without cell were measured. With a cell, near at 1300 Hz (arrow) about 10 nm membrane fluctuation was detected.

3.5 ATP Depletion Abolishes All Membrane Fluctuations

Finally, we test the role of ATP using ATP depletion agent. We found that ATP depletion abolished all membrane fluctuations. This is because ATP depletion stiffened cytoskeleton.

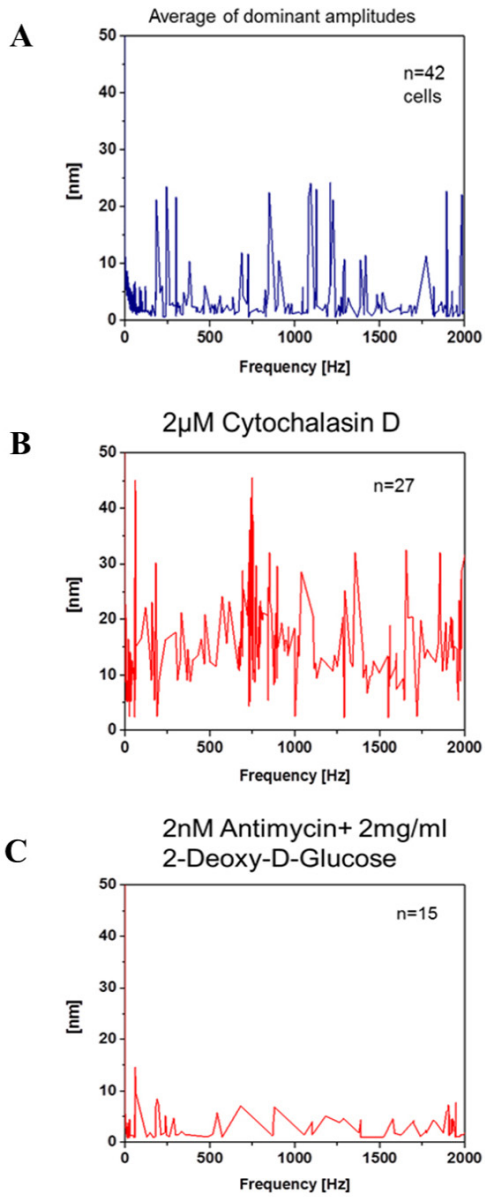

Fig. 5 Assessed cell surface dynamics. Characteristic frequencies of intact (A), actin depolymerized (B), and ATP depleted (C) endothelial cells

4. Conclusion

We developed a non-contact method to measure cell surface fluctuations through measurements of resistance between a micro-electrode tip and the cell membrane. We found that Endothelial cells exhibit local membrane fluctuations of ~20 nm at characteristic frequencies, actin depolymerization causes increase in fluctuation amplitude up to 2 times at all frequencies, and ATP depletion abolishes all membrane fluctuations. Cell surface fluctuations are possibly dependent on the extracellular and intracellular conditions, such as temperature, physical properties of cell culture substrates, and plasma membrane tension. Our system provides enhanced tools to test effect of these conditions on cell surface dynamics.

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