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Destabilization induced by electropermeabilization analyzed by atomic force microscopy

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ABSTRACT

Electropermeabilization is a physical method that uses electric field pulses to deliver molecules into cells and tissues. Despite its increasing interest in clinics, little is known about plasma membrane destabilization process occurring during electropermeabilization. In this work, we took advantage of atomic force microscopy to directly visualize the consequences of electropermeabilization in terms of membrane reorganization and to locally measure the membrane elasticity. We visualized transient rippling of membrane surface and measured a decrease in membrane elasticity by 40%. Our results obtained both on fixed and living CHO cells give evidence of an inner effect affecting the entire cell surface that may be related to cytoskeleton destabilization. Thus, AFM appears as a useful tool to investigate basic process of electroporation on living cells in absence of any staining or cell preparation.

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1. Introduction

Electropermeabilization (EP) consists in the local application of electric field pulses to cells and tissues which renders the plasma membrane transiently and locally permeable to non-permeant molecules [1]. This physical method is used in clinics to potentiate the cytotoxic effect of anticancer drugs (electrochemotherapy) [2,3] and has great promise for gene transfer (electrogenotherapy and vaccination) [4–6]. In 2012, more than 3000 patients have been treated by electrochemotherapy, while 8 clinical trials were ongoing.

However the electric field effects are still poorly understood at the molecular level [7]. Indeed, membrane permeabilization had only been indirectly studied by the entry of fluorescent or radioactive markers and countable molecules into cell populations during and after pulse application [8,9], or by transmembrane potential measurements [7]. Permeabilization has been described as localized at the sides of the cell facing the electrodes [10], which is explained by the change in membrane polarization at these two polar positions regarding electric field (hyperpolarization facing the anode and depolarization facing the cathode) [11]. Furthermore, physical mechanisms at the molecular level described by numerical simulation have shown water wire in lipid bilayer during pulse application [12], indicating lipid abilities to change their transmembrane orientation, in agreement with both theoretical [13] and experimental studies [14]. Finally, lipid loss inducing several membrane alterations (pores, tubules and vesicles formation) has been observed on giant unilamellar vesicules submitted to electric pulses [15]. All these observations tend to prove the existence of lipid disorganization due to electric field application and different ways of membrane destabilization. However, most of these studies used fluorescent dyes that can create some artifact as they are most of the time charged, while simulations were undertaken with non-relevant electrical conditions regarding biological application. Consequently, the need of data recorded directly at the single cell level without any staining or preparation was clearly felt and required further measurements. In this context, our challenge was to visualize the effects of membrane destabilization resulting in permeabilization to small molecules and to perform direct quantitative biophysical measurements using atomic force microscopy (AFM).

Abbreviation: AFM, Atomic Force Microscopy; EP, Electropermeabilization; YM, Young Modulus; CHO, Chinese Hamster Ovary Cell; QI™, Quantitative Imaging™

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During the last decades AFM has emerged as a valuable tool to explore the cell membrane biophysical properties [16,17]. We have chosen to use its force spectroscopy mode to measure EP effects on cell membrane elasticity at the single cell level. We used the innovative Quantitative Imaging (QI™) mode from JPK [18], which is a high speed and resolution force volume mode designed for soft and loosely immobilized samples. Based on the force volume mode measurement, QI™ mode allows to record images at a high speed without exerting lateral pressure on the sample [19]. We worked with Chinese hamster ovary (CHO) cells with electrical condition which was used for gene transfer [20]. It is a commonly used model for electroporation study [9] that has not been much studied by AFM [21]. We studied both fixed and living cells to access the different steps of membrane electropermeabilization. Force measurements allowed quantifying and describing along time the effect, and height images of living cells which gave first assumptions on effect of electric field on membrane shape (Fig. 1.A). Nanomechanics revealed that electric field provokes a decrease in YM of plasma membrane by 40% and that the effect observed may be longer than showed by fluorescence imaging after pulse application. This paper states that AFM can be used to study electroporation phenomenon at the single cell level, and can be a helpful tool to investigate at the basic level of electro-destabilization.

2. Materiel and methods

2.1. Sample preparation

2.1.1. Fixed cells

150,000 Chinese hamster ovary cells (wild type ATCC) were grown during 48 h on a coverslip in minimum Eagle's medium (MEM 0111, Eurobio, France) supplemented with 8% fetal calf serum (Lonza Group Ltd, Switzerland), and incubated at 37 °C in humidified atmosphere with a 5% CO_2 incubator. Prior to electrical pulse application, cells were washed 3 times with phosphate-buffered saline (PBS) 1× (Invitrogen, USA). 1 mL of pulsation buffer (PB) at 4 °C (10 mM K_2HPO-4/$KH_2PO_4^-$ buffer, 1 mM $MgCl_2$, 250 mM sucrose, pH 7.4) was added, electrodes were placed in contact with glass surface and pulses delivered. Immediately after pulse application, PB was removed, and 1 mL of 4% paraformaldehyde solution (Sigma-Aldrich, Saint-Louis, MO) in PBS 1× was added, and coverslip was placed at 4 °C for 30 min.

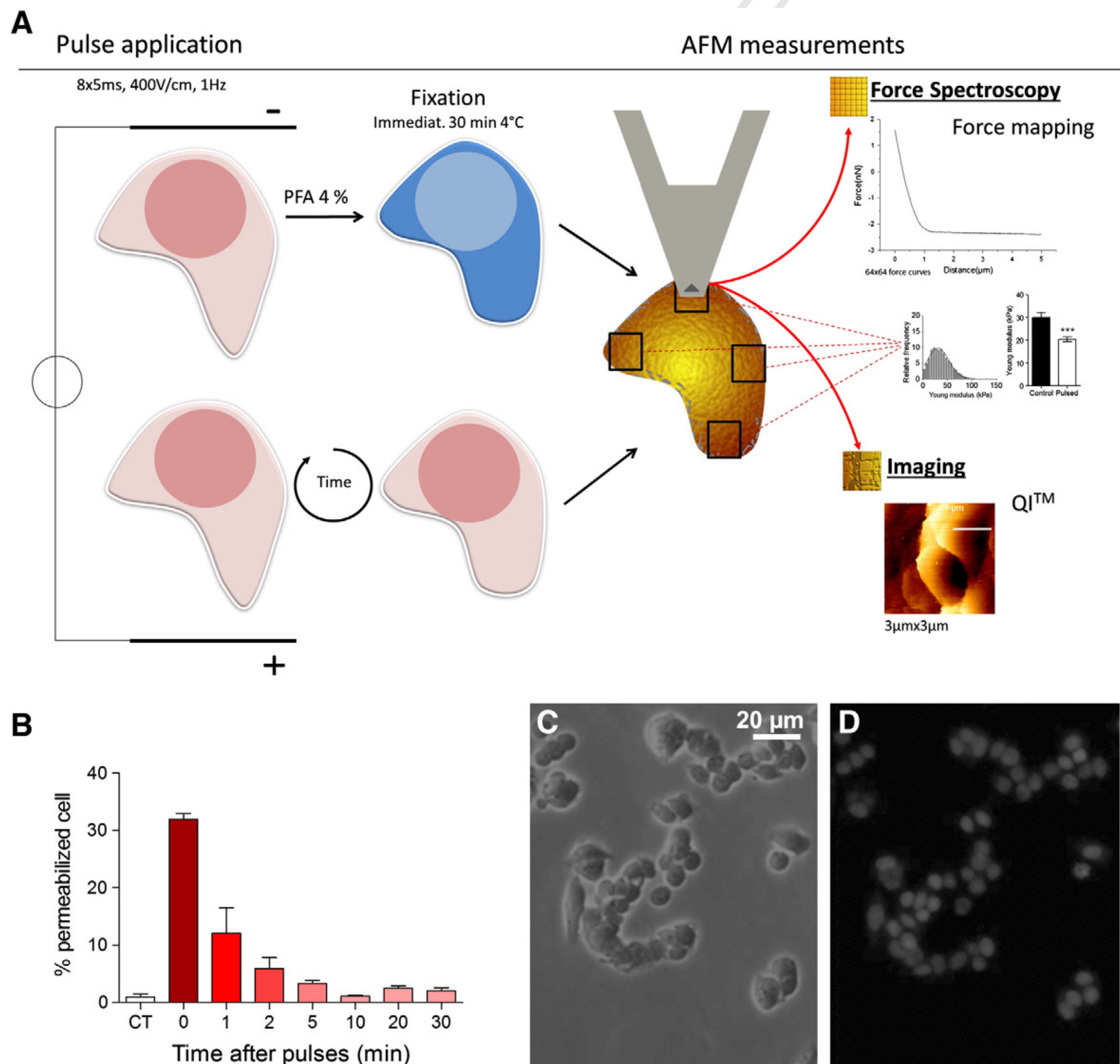

Fig. 1. Experiment outline and permeabilization control. (A) Experiment outline. Cells are pulsed and then either fixed or kept alive to be imaged and measured by AFM. (B) Permeabilization and membrane resealing along time. CT is the control representing cell not subjected to electric pulses. The number of PI positives cells is directly related to the membrane permeabilization and decreases along time. (C) and (D) Phase contrast and fluorescence image of cell 5 min after pulse application with PI.

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