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Diode Character of Local Conductivity of Human Buccal Epithelial Cell Membranes

N. A. Torkhov and A. A. Mosunov

Sevastopol State University
33, Universitetskaya Str., Sevastpol, 99053, Russian Federation
trkf@mail.ru, aamosunov@sevsu.ru

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Abstract: *AFM methods revealed unilateral (diode) local electrical conductivity of the membrane of human buccal epithelium cells at the nanoscale and its correlation with the topology of micromechanical properties.*

Keywords: *human buccal epithelial cells, conductivity, AFM methods.*

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1. Introduction

Intensive development of bioelectric integrated human systems based on the propagation of constant [1] and variable [2] electrical signals in his tissues requires study of the electrical properties of the body at the cellular level. Great information content regarding the influence of various physical and mechanical factors (signals), good accessibility and non-traumatic reproduction of sampling, simplicity and cheapness of sample preparation makes buccal epithelium cells of the human oral mucosa a convenient biological object for conducting such studies [3]. The research results can serve as a source of important diagnostic and prognostic information about socially significant human diseases, stress effects, the influence of environmental factors and xenogenic intoxication, pharmacology [1, 4].

Currently, one of the common probe methods for local measurement of currents of various channels of biological membranes in static mode is the so-

called “patch-clamp” (patch–clamp [5]) method, which is based on the fact that under certain conditions, the open end of a glass microelectrode pipette can form a sufficiently tight contact with the surface of the cell membranes with a resistance of 10^9 Ω. For the development of this method, E. Neer and B. Sakman were awarded the Nobel Prize in 1991.

One of the advantages of this method is that the size of the tip of the measuring probe (a metal microelectrode, or a glass micropipette filled with an electrolyte solution), in contrast to the “potential fixation” method previously used at the meso-level (the diameter of the pipette hole ~60 μ*m* [5]), had a significantly smaller size of the tip of the metal needle, or output the holes of the micropipette are of the order of one micrometer, and, consequently, a smaller S_c contact area. This made it possible more accurately investigate the electrophysical (for example, the electrostatic resting membrane potential) and conductive (electric currents at the level of 10^{-12} *A*) properties of membranes, and the behavior of various transport processes in them at the micron (3–10 μ*m*) level, determined in this case by the accuracy of the spatial positioning of mechanical micromanipulators. The positioning accuracy of modern mechanical micromanipulators usually varies from units to tens of micrometers, which in most cases is commensurate with the external dimensions of cells and for this reason allows us to obtain only some averaged values of the electrophysical characteristics of cell membranes at the micron level. In general, this method allows conducting research within the framework of classical electrophysiological concepts, as well as investigating the effect of drugs when they are directly brought to the membrane.

According to the laws of electrostatics, if the dimensions of the conducting probe and the studied object (cell) are commensurate, all areas of the cell will participate in the conductivity, which makes it difficult to interpret the results. In other words, the entire membrane will participate in conducting of electric current as a kind of structured conductor, in the conductivity of which all its organelles will be involved.

The high spatial and functional resolution of AFM methods allows, unlike the methods described above, obtain accurate values of geometric and functional parameters in 3D projection at the nano- and atomic level. In this case, when the sizes of electrical contacts vary from units to tens of nanometers, the electrical effect does not cover the entire object, but due to the effect of spreading currents only the local area around the contact, usually not exceeding the size of most cellular organelles.

In addition, AFM methods have a large set of functional characteristics measured in a wide range of temperatures (for example, from –4 °*C* to >100 °*C*)

and pressures (up to ~10^{-3} Top), as well as in various gaseous and liquid media: relief, phase contrast, tribological properties, spatial distribution of micromechanical and electrophysical characteristics on direct and alternating currents, galvanic and magnetic effects.

All these AFM capabilities make it possible to carry out local controlled effects on biological objects not only at the cellular, but also at the molecular level, which opens up wider opportunities and prospects at new qualitative and quantitative levels to investigate the conductive properties and mechanisms of functioning of various intercellular and membrane channels, changes in the membrane potential and maintenance of electrical stability of the cell, physicochemical mechanisms of various receptors action, nature of the transformation of mechanical stimulation into electrical signals (for example, in cardiomyocytes and fibroblasts), nature of mechanoelectric feedback through highly permeable contact zones of membranes and much more.

Nevertheless, despite the obvious successes in this direction, the volume of experimental data on the electrophysical nature of cell membranes and their reactions to local external electrical stimuli is still extremely insufficient.

In this regard, the purpose of this work is to study by atomic force microscopy (AFM) the conducting ability of the membrane of living human buccal epithelium cells.

2. Materials and methods

The object of the study was human buccal epithelial living cells (hereinafter referred to as cells) obtained by liquid cytology. This method included mechanical sampling (scraping) from the inner surface of the cheek of the mucous membrane oral cavity, washing of the scraping in a phosphate buffer (3.03 *mM* phosphate buffer with the addition of 2.89 *mM* calcium chloride with a volume of 5 *ml*, pH 7.0), placing this mixture in a test tube and separating its contents in a centrifuge for 5 minutes with an acceleration of 1700 g (5000 min^{-1}), taking aliquots of a buffer solution containing suspension of living buccal epithelial cells. The epitaxial structure of silicon of p-type conductivity p– p+– Si{111} with the size of irregularities <20 pm was used as a substrate material.

After placing the aliquot on the epitaxial surface of silicon Si{111}, it was dried in air at normal atmospheric pressure and temperature $T = 40\ ^{\circ}C$ for <10 minutes. The living cells in the aliquot were naturally deposited on the epitaxial silicon surface and preserved on it in this form for 3–4 hours after evaporation of the main amount of moisture. The presence of an adsorption layer of buffer solution on the cell surface maintained their viability during a relatively long stay in the air under normal conditions (NU).

Studies of the geometry of the relief (Fig. 1) of the surface of cell membranes and their electrical conductivity – the electron-hole spreading current *Ipr* (hereinafter referred to as the spreading current Ipr) (Fig. 2a and Fig. 3a) was carried out in the air at NU using the NTEGRA-AURA AFM with a scanning object table in contact scanning mode and a resolution of 300×300 points providing constant mechanical and electrical contact of the needle tip of the cantilever with a constant pressing force to the surface of the "Molecular Structure of matter" conducted on the basis of the Center for Collective Use Sevastopol State University. Conductive HA-FM/W2C cantilevers with a radius of rounding of the needle tip r~35 *n*m were used as a measuring probe. The cantilever was grounded.

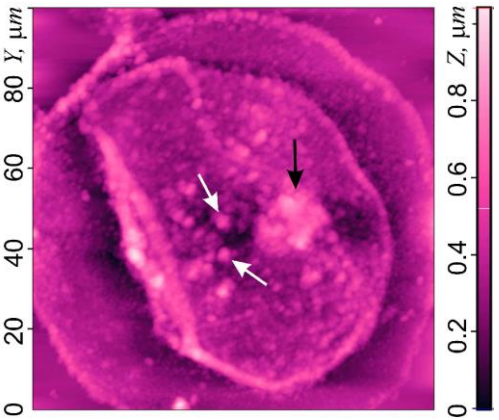

Fig. 1. Raster AFM image of the relief of a living human buccal epithelium cell

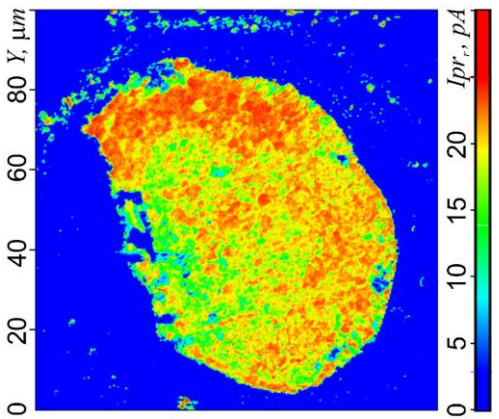

Fig. 2*a*. Raster AFM image of the distribution of spreading currents *Ipr=Ipr(x;y)* on the surface of the membrane of a living human buccal epithelium cell with a reverse displacement U_r =–3 V

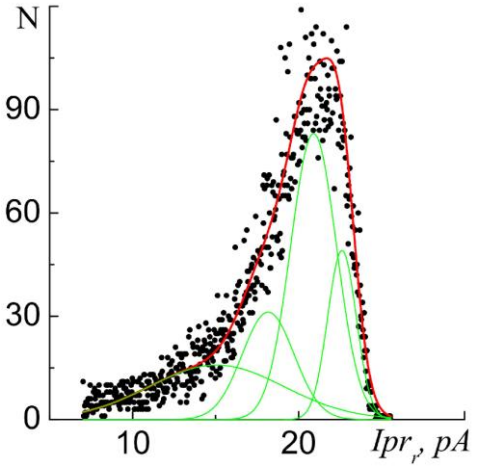

Fig. 2*b*. The histogram *N=N(Ipr)* presented in Fig. 2a spreading currents

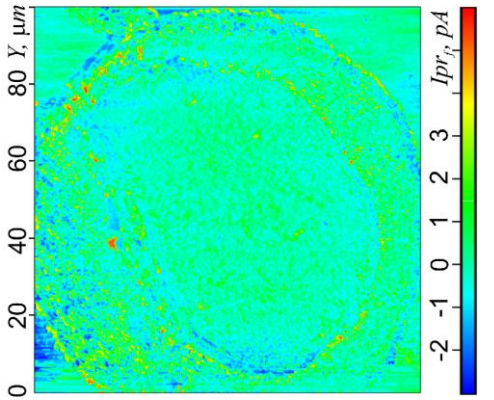

Fig. 3*a*. Raster AFM image of the distribution of spreading currents Ipr=Ipr(x;y) on the surface of the membrane of a living human buccal epithelium cell with a forward displacement Ur=+3 V

3. Results

The location of the cell nucleus (Fig. 1, black arrow) and the surrounding organelles (Fig. 1, white arrows) are clearly visible on the surface of the membranes. At the same time, the shell surface itself is not smooth, but has a sufficiently developed relief with a well-defined morphological structure and mechanical organization [6, 7].

Studies have revealed unilateral heterogeneous conductivity of the membrane of human buccal epithelium cells. To determine the type of conductivity (ionic, electron or hole), further research is necessary.

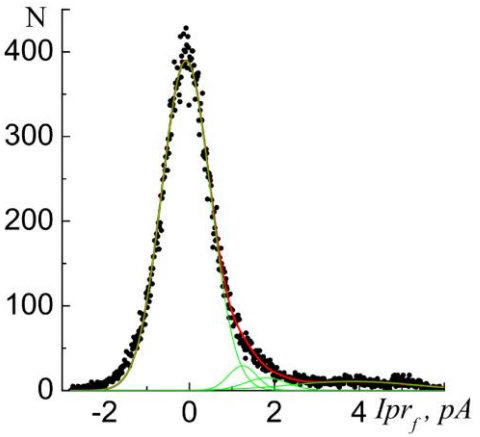

Fig. 3*b*. Histogram *N=N(Ipr)* of the spreading currents shown in Fig. 3a

The histogram of the distribution N = N(Ipr_r) shows that the membrane is formed by regions with different electrical conductivity (Fig. 2b), which are formed by a set of smaller areas with similar values of spreading currents $Ipr_f=Ipr_f(x,y)$. Studies have shown that the topology of these sites coincides quite well with the topology of local terrain irregularities (Fig. 1) and the distribution of adhesive forces over the membrane surface $F_{adh}=F_{adh}(x,y)$, as well as the work performed by them $A_{adh}=A_{adh}(x,y)$ [7]. A more detailed analysis showed that ion nanochannels penetrating the membrane have several times higher conductivity compared to their surrounding surface. At the same time, as follows from Fig. 1, the heterogeneity of membrane conductivity partially correlates with the nature of the location of some cell organoids. This may indicate the existence of an electrical connection of such organoids with the cell membrane.

Comparison of raster images of spreading currents revealed one-sided electrical conductivity of the membrane. So, for $U_r<0$, the average values of currents $\langle Ipr_r\rangle$=20.8 *pA* (Fig. 2a) significantly exceed the values of $\langle Ipr_f\rangle$=0.01 *pA* for $U_f>0$ (Fig. 3a).

Studies have shown that, depending on the state of the cell, the conductivity of different areas of the cell membrane can vary within a sufficiently wide range, which can be used to assess the state of the cell.

The research was conducted in accordance with the principles of the Helsinki Declaration. Permission to conduct studies with buccal epithelium sampling was obtained by the Ethics Committee of Sevastopol State University (Study No. 3, July 15, 2021).

Buccal epithelium was collected in accordance with the rules for conducting research on human material in the Russian Federation. All subjects have given written informed consent.

Thus, the method of AFM spreading current revealed unilateral (diode) local electrical conductivity of the membrane of human buccal epithelium cells at the nanoscale and its correlation with the topology of micromechanical properties.

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Information about the authors

Nikolay A. Torkhov, Leading Researcher at the Research Laboratory "Flight Dynamics and Control of Unmanned Aerial Systems" of the Institute of the National Technology Initiative, Sevastopol State University, Sevastopol, Russian Federation. ORCID: 0000-0001-8902-6319.

Andrey A. Mosunov, Researcher at the research laboratory "Molecular and Cellular Biophysics", Sevastopol State University, Sevastopol, Russian Federation. ORCID: 0000-0002-1390-5832.