Multichannel patch-clamp system with three-dimensional porous alumina structure on planar chip
New generation of the patch-clamp systems

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Abstract

Patch-clamp technique becomes a powerful method in studying cell excitability, as well as mechanisms of their regulation by different metabolic factors, pharmacology and drug candidates.
The main idea of this study is employing of unique properties of amorphous porous alumina layers to perform three-dimensional architecture of recording site. Increased throughput is achieved by automation and parallel measurements on several cells. Multisensor-systems on the basis of nanoporous anodic alumina: highly ordered arrays of nanoholes with several hundreds down to several tens of nanometer diameter; substrate thickness within 0.005-1 mm patterned down to ~20-50 nm, wide range of compositions providing different electrophysical and optical properties, flexible, reconfigurable with the possibility of embedding in mobile manufacturing line, enables mass production without the use of expensive lithographic tools.

Key words: patch-clamp technique, nanoporous anodic alumina, multisensor-systems

1. Introduction

The patch-clamp technique has revolutionized the study of membrane physiology, enabling unprecedented resolution in recording cellular electrical responses and underlying mechanisms. Patch-clamp technique becomes a powerful method in studying cell excitability, functions of ionic channels as well as mechanisms of their regulation by different metabolic factors, pharmacology and drug candidates [1-5]. Several recording configurations of the patch-clamp technique enable investigation of macroscopic currents of entire cells as well as elementary single-channel currents in microscopic membrane pieces (patches) [6-8]. Traditional patch-clamp technique uses a micropipette with a relatively large tip diameter. Most patch-clamp measurements are performed in a voltage-clamp mode, where the membrane potential is held at a given level, while the current through ionic channels is displayed. In addition, the patch electrodes are successfully used in current-clamp studies, predominantly with entire cells, for recording changes in membrane potential in response to injection of command current pulses. The amplitudes of ionic currents studied by means of the patch-clamp technique range in most cases from 0.1 pA to 10 nA.

2. Materials and Method

2.1. Traditional patch-clamp technique

The patch-clamp technique uses a micropipette with a relatively large tip diameter. The single micropipette is pulled from glass capillary. Micromanipulation and microscopic observation are

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required to move the solution-filled pipette tip as close as possible towards the cell membrane. The microelectrode is placed next to a cell, and gentle suction is applied through the microelectrode to draw a piece of the cell membrane (patch) into the microelectrode tip (Fig. 1). Ion channels: Na+, K+, Ca++, Cl- channels potential-gated channels ligand-gated channels mechano-sensitive channels.

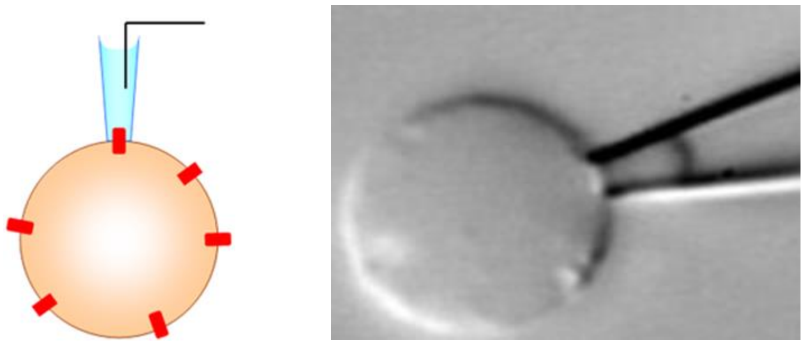

Figure 1. Patch clamp technique principle

Technique application - Ion channels drug candidates screening, Ion channels toxins screening, Ion channels pathology screening, Ion channels related processes screening [9-12].

In order to form the cell-attached mode, a pipette tip is placed on the surface of the cell, forming a low resistance contact (seal) with its membrane. Slight suction applied to the upper end of the pipette results in formation of a tight seal with a resistance of 1 to 100 GOhm. Such a seal with a resistance in the range of gigaohms is called giga-seal. Formation of a giga-seal is extremely important for reduction of leakage current during recordings.

2.2. Voltage clamp and current clamp

Most patch-clamp measurements are performed in a voltage-clamp mode, where the membrane potential is held at a given level, while the current through ionic channels is displayed. In addition, the patch electrodes are successfully used in current-clamp studies, predominantly with entire cells, for recording changes in membrane potential in response to injection of command current pulses (Fig. 2). The amplitudes of ionic currents studied by means of the patch-clamp technique range in most cases from 0.1 pA to 10 nA. Designed for this purpose, the basic recording circuitry of the patch-clamp amplifier consists of a standard current-to-voltage converter with a large feedback resistor and a differential amplifier (Fig. 3) [13]. The current-to-voltage converter forces the pipette potential to follow the applied command potential. The recorded pipette current flows through feedback resistor producing a measured voltage drop. Typical feedback resistor values are in the range of 5 MOhm to 50 GOhm.

Institute of Physics of NASB and Belarusian State University, Biophysics Department, cooperate in the area of investigation of conventional patch-clamp including: cell culturing, cell patch-clamping, chemicals application, data handling, data analysis.

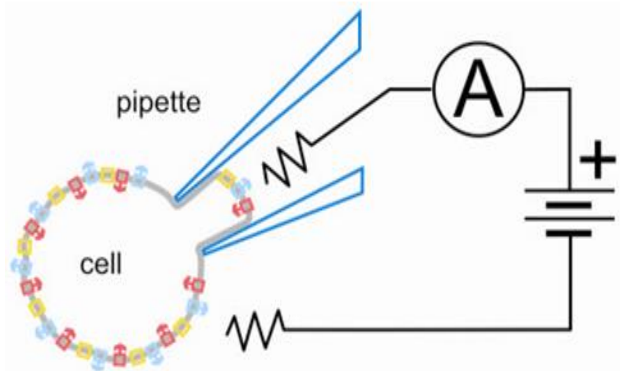

Figure 2. Schematic Patch clamp technique based the current through ionic channels

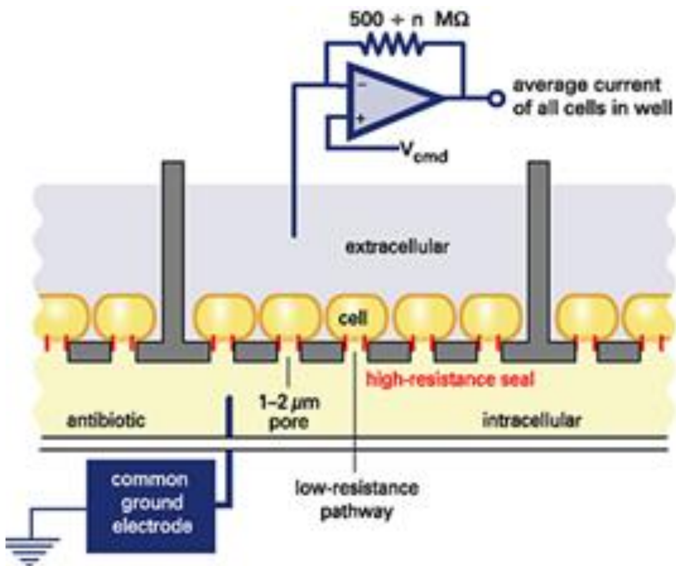

Figure 3. The basic recording circuitry of the patch-clamp amplifier [13]

3. Multichannel Patch-clamp Systems

The main idea is employing of unique properties of amorphous porous anodic alumina layers to perform three-dimensional architecture of recording site. Anodic alumina (AA) is one of prospective composition materials, applied for the creation of devices and technical systems, the functioning of which is determined by a nanostructure [14,15]. Such a material, obtained by the electrochemical oxidation of aluminium in acid electrolytes, possesses wide potential capabilities [16]. AAO films have a regular periodical structure of nanopores, which are perpendicular to the substrate surface. At the same time the diameter of pores and the distance between them can be regulated in wide ranges (from 10 nm up to hundreds of nm) by selection of technological process. AAO has high electromechanical parameters. The units and various components, made of this material, have the high precision, planar and volumetric configurations with blind and through holes, slots and dimples. The technological process is based on the integral methods and electrochemical operations of growing and etching of the oxide and aluminium with the subsequent putting on the thin metallic coatings. The diversity of forms of units made of AAO stipulates the opportunity for the creation of various microsensors with the wide functionality [17-19].

For the last ten years the amount of works in various areas of science and technology, especially in the region of nanotechnology, with the use of AAO has increased up to tens of thousands. About a hundred well-known scientific groups and single researches paid their attention to the AAO uniqueness and proposed their variants for creating the constructions and devices, based both on nanoporous AAO and aluminium oxide thin films with periodical system of nanopores. As an example, the following variants can be given: structures with AAO thin films, AAO nanoporous molecular membranes, nanoporous matrixes from AAO as soluble former blocks for construction of the photonic crystals, modified nanoporous AAO matrixes as sensitive sensor elements [20-24].

In comparison with other technologies based on self-organization, aluminum oxide is prospective for the formation of nanocapillary system matrixes in relation to the demands of high quality magnetic and magneto-optical materials and sensors based on them [25].

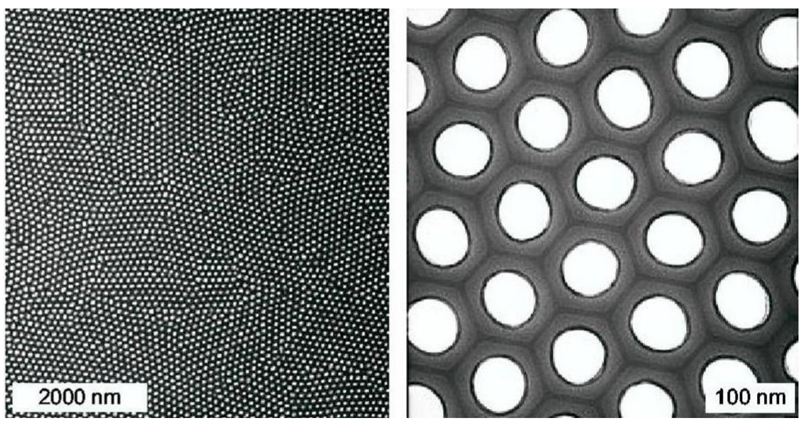

Figure 4. Microphotography's of surface porous alumina

3.1. Parallel measurements on several cells

Increased throughput is achieved by automation and parallel measurements on several cells. Combining microtechnology and electrophysiology opens the way to a new generation of chip-based patch-clamp devices. Planar patch-chips can be easily integrated into microfluidic circuits [26]. The chip separates two fluidic compartments connected by an electrical and fluidic pathway through the micro-aperture. Electrical contacts are integrated on each side of the chip. Self-positioning of cells on the micro-aperture is generally achieved by hydrodynamic forces, i.e. by applying a negative pressure gradient to the backside of the chip.

3.2. Development of oxide-aluminum technology and nanodevices based on it.

IP NASB has acquired great experience in the field of the development of multisensor systems based on Al_2O_3 matrixes [27]. The mentioned sensors are sensitive to the variations of optical, magnetic, electrostatic and other fields and assigned for the automatic control of operation of complex systems. An important advantage is the opportunity of their exploitation in active zones with the extreme environmental conditions (high radiation, low or high temperatures and so on), for example in the operating zone of the reactor [28].

The preliminary researches on the stability control substrates of anodic aluminum oxide to a physiological saline solution, which contains cells and investigated, showed practical immutability of material substrates with multi-day keeping of substrates in such a solution.

3.3. Technical advantages for multisensor-systems on the basis of nanoporous anodic Al_2O_3 is [29]:

- Highly ordered arrays of nanoholes with several hundreds down to several tens of nanometer diameter; substrate thickness within 0.5 - 1 mm patterned down to ~20-50 nm;
- Wide range of compositions providing different electrophysical and optical properties;
- Flexible, reconfigurable with the possibility of embedding in mobile manufacturing line;
- Enables mass production without the use of expensive lithographic tools, such as electron beam exposure system.

3.4. Multichannel Patch-clamp Systems with Three-dimensional Porous Alumina Structure on Planar Chip

- Suitable multichannel patch-clamp system is proposed to be developed on planar chip with increased throughput in testing.
- High-throughput is achieved by application of novel architecture and materials. The main idea is employing of unique properties of amorphous porous alumina layers to perform three-dimensional architecture of recording site.
- It is proposed to develop new concept and design of the planar patch-clamp system, and ready-to-use prototype of the planar patch-clamp chip (Fig. 4).

High-throughput multichannel patch-clamp systems in great demand in high-throughput screening in industrial ion channel drug development, in clinical cytology and in academic research.

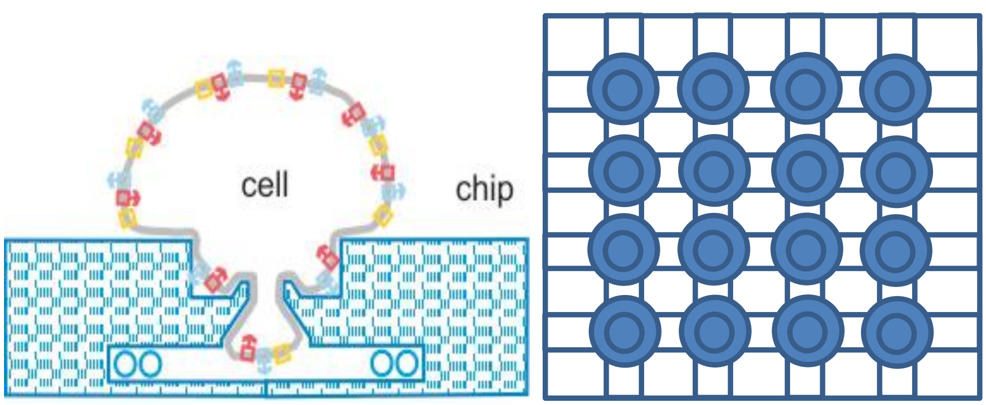

Figure 4. Schematic view the 4X4 cells of the planar patch-clamp chip

3.5. Multichannel Patch-clamp Systems

– The architecture of the chip will provide four clusters with 4x4 operation channels in each claster.

- Every channel will contain several measuring site. The sites will separate two fluidic compartments connected by an electrical and fluidic pathway through the micro-aperture.
- Every channel will enable fluidic interface to perform suction for giga-seal and whole-cell mode formation. Every fluidic compartment will include metal electrode for cell membrane currents or potential fixation.
- The shape of recording site will be performed to meet the demands of high-throughput giga-seal formation.

Conclusions

Modern automated planar multichannel patch-clamp techniques are promising screening systems for various areas of biotechnology and medicine. Different patch-clamp techniques based on the electric potential/current control/recording allow flexible approach for ion channels investigation. Micro-electro-mechanical systems based on nano-porous anodic aluminum oxide substrates can be used as flexible platform for cell sensors. We propose a planar multichannel patch-clamp chips based on the anodic aluminum oxide substrates.

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