Electro-physical properties of cell monolayer on nanocomposite coating based on carbon nanotubes LB-films

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1. Introduction

Ordered array of carbon nanotubes (CNT) and nanoporous anodic alumina (AOA) samples possess unique properties. Nanocompositeses fabricated on base of these materials as sensitive nanoelements for measuring technique open up huge possibilities in a immunoanalysis and in investigations of living processes on a cell level. An impedance analysis of growing cells by interdigital capacity sensors covered by Langmuir-Blodgett (LB) films with multi-walled CNT provides information on cell number, cell morphology, cell-cell adhesion and cell-matrix adhesion. The impedance analysis used for measurement of kinetics of cellular state during long period of time and these measurements are carried out on chosen one frequency [1–5]. In our work we tried to reveal the useful of cellular electric properties determination in a same frequency range.

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The goal of the paper is to study a capacitive response on cellular monolayer depending on the cellular physiological state and to propose a new method of oxidation-reduction potential of cells based on phenomenon of spontaneous polarization of nanosensor coatings with CNT.

2. Materials and methods

In our work we used interdigital capacity sensors of "open capacity" type. Sensor electrodes have been arranged symmetrically along the square sides. An electrode dielectric coating consists from the nanoporous anodic alumina covered by LB-film with multi-walled CNT. CNT were previously modified by carboxyl or hydroxyl groups. Biosensitive coatings contained rare earth elements (Ce and Sm) at different concentration.

Rat C 6 glioma cell line was purchased from the Cell Culture Collection of the Institute of Cytology (St. Petersburg, Russia). The sensor surface was coated with C 6 glioma cell suspension in Eagle's Minimum Essential Medium (MEM) (Sigma, USA) supplemented with 10 % fetal bovine serum (Sigma, USA) and 100 g/ml gentamycin (Belmedpreparaty, Belarus). Cells were routinely grown in monolayer on sensor in tissue culture dishes at 37 ^{O}C in a humidified atmosphere of 5 % CO_2 and 95 % air. After removing culture medium cell monolayer was washed with Erla buffer saline solution or MEM at 37 ^{O}C. Versene (Sigma, USA) was used to lift cells from sensor surface.

(a) (b)

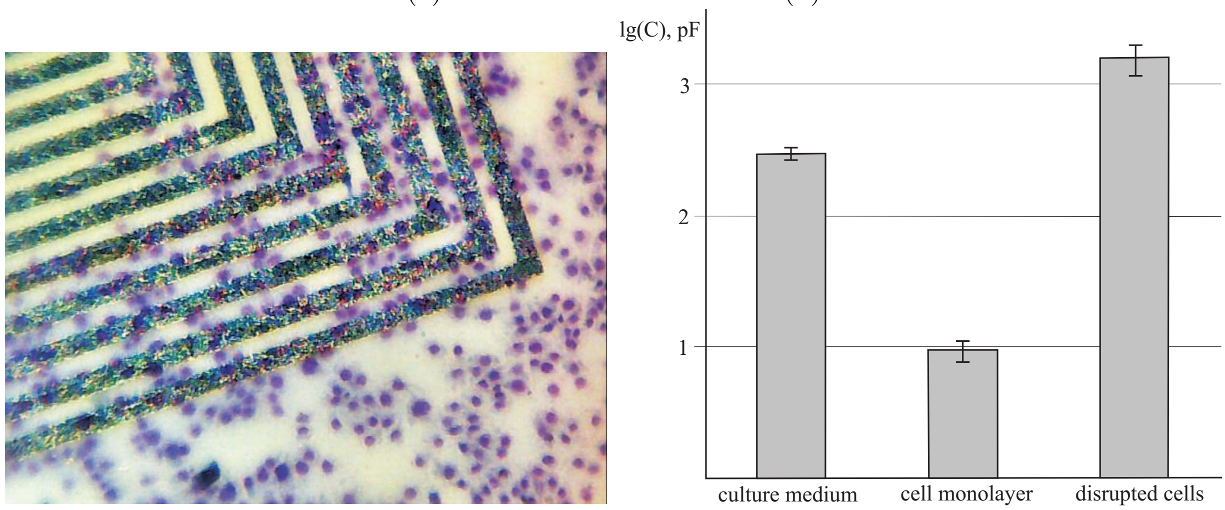

FIG. 1. Detection of cellular physiological state

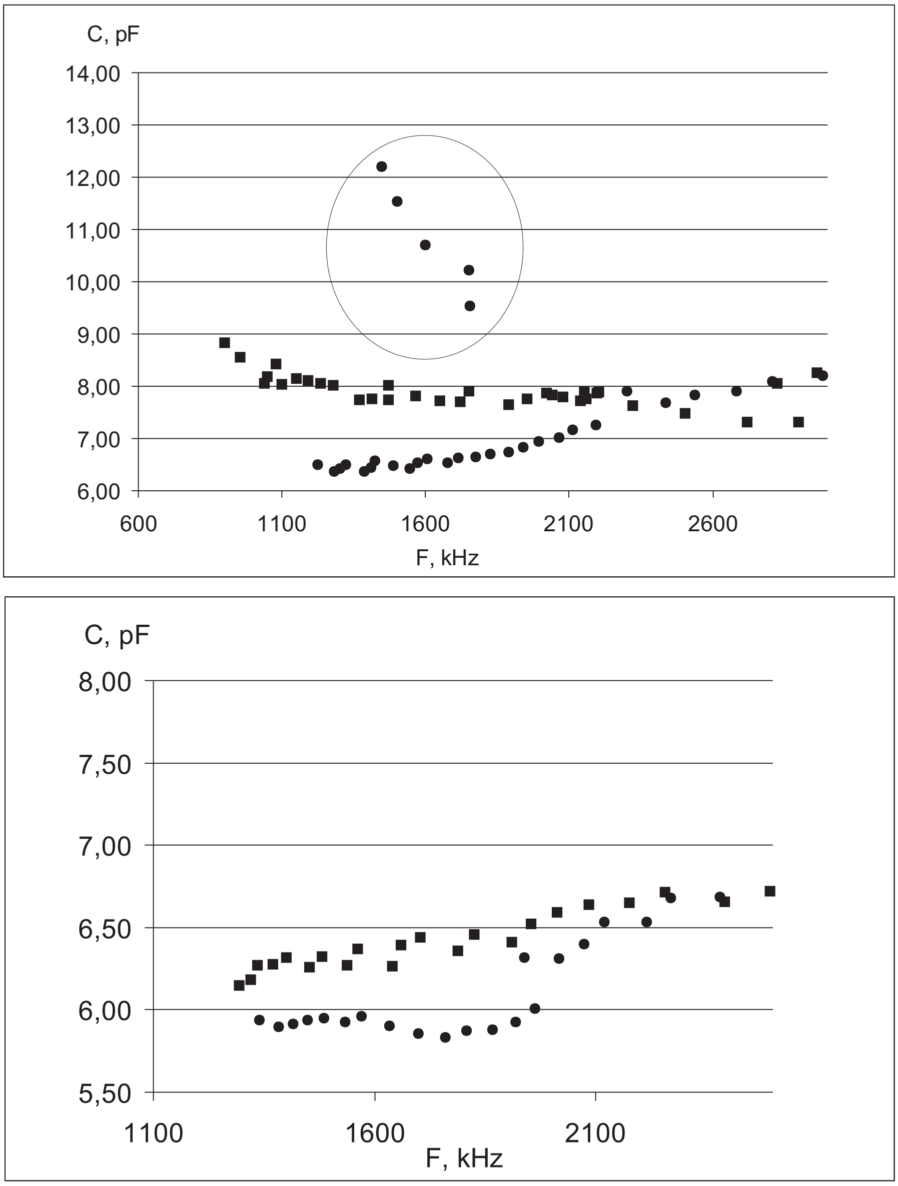

FIG. 2. Spontaneously polarization of cell monolayer: Fulfilled circles - direct branch, fulfilled squares - return branch

3. Results and discussion

It was found that a coating cell monolayer decreases the sensor capacity relative to balance buffer saline solution (neutral pH) (fig. 1). Therefore one can say about effect of electric field shielding by the cell monolayer. The cell disruption by them coloration or lysis in distillate water results in sharp increase of capacity of the sensor with cell monolayer from about 10 to 1500 pF (picofarad) in frequency range from 1000 to 3000 kHz. It is connected with cell death and

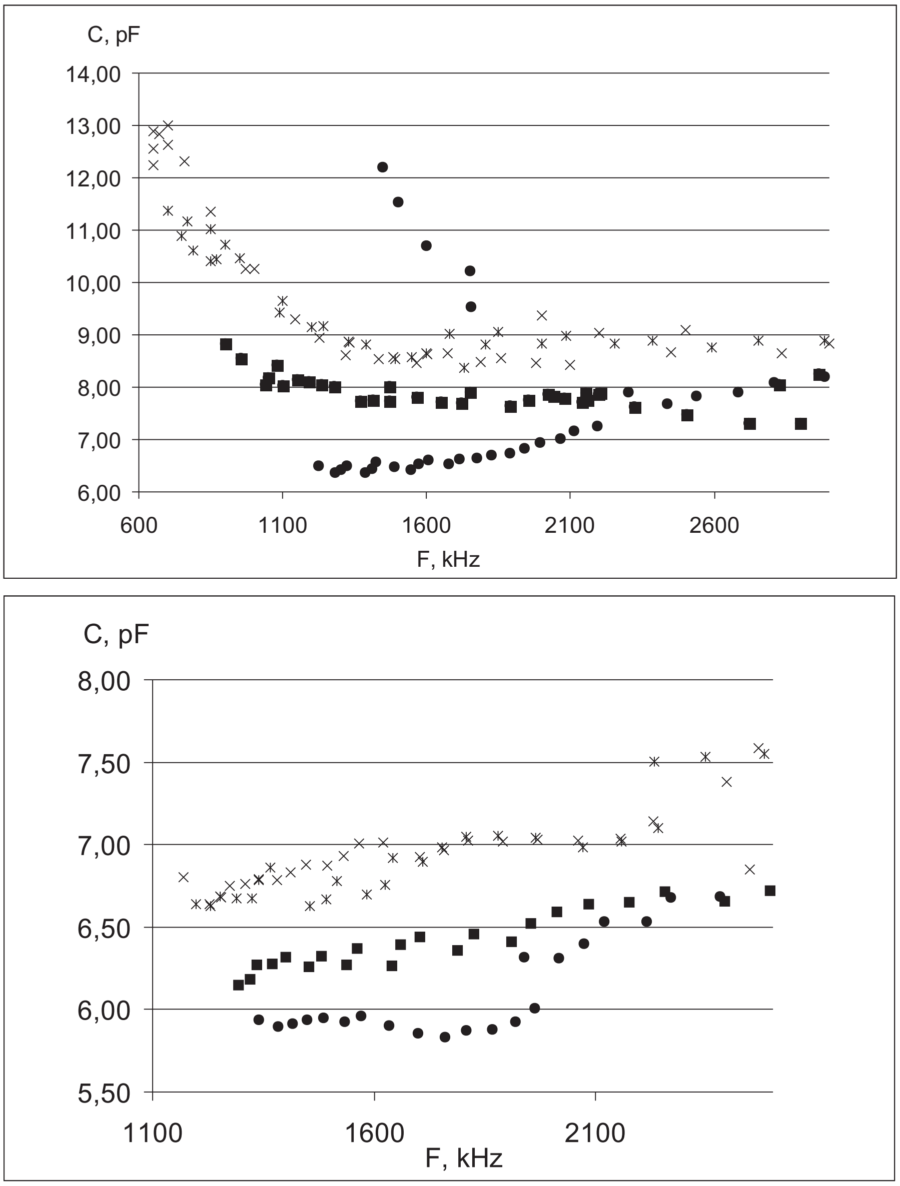

FIG. 3. Spontaneously polarization of cell monolayer disappears at menadione addition in 1×10^{-4} mol/l concentration - without menadione addition: fulfilled circles - direct branch, fulfilled squares - return branch, - at menadione addition: crosses - direct branch, asterisks - return branch.

release of ions into water. The cells lift from the sensor surface by versene returns the initial, without cells, sensor indications.

It was shown that capacity characteristics of cellular monolayer reveal the spontaneous polarization as an asymmetry of branches of cyclic frequency characteristics in frequency range from 1000 to 2000 kHz. This asymmetry can be connected with either CNT dipoles reorientation and alignment with formation of dipole-ordered domain structure, that was observed for sensors without cellular monolayer, or reorientation of electrical charge in cellular membrane.

An anticancer activity of menadione has been demonstrated in a number of works [6–8]. The anticancer effects of menadione could be determined by its ability to induce reactive oxygen species (ROS) generation in cells. Besides this it was shown that menadione molecules can influence on gap junction cellular communication, Ca^{2+} intracellular level and can modify activity of some kinases and phosphotases [9–11]. To investigate menadione effect on cellular physiological state we have performed a series of experiments based on cyclic dielectric spectroscopy method.

It was shown previously by methods of chemiluminescent and fluorescent analysis that the menadione action on C 6 glioma cells leads to ROS generation both in cells and extracellular medium [12, 13]. The increase of capacity of cellular monolayer at 1–2 pF in the frequency range from 1000 to 3000 kHz was observed after menadione addition in 1×10^{-4} mol/l concentration. This effect was assumed to be associated with extracellular ROS production. Besides this, spontaneous polarization was disappeared after the menadione action. The added menadione molecules are likely embedded in cellular membrane. Some disordering of cell membrane structure results from this embedding because the spontaneous polarization cannot be observed practically.

The existence of special frequency ranges in which oscillations of capacity characteristics occur is another phenomenon founded during our experiments. There are two branches: 700-1000 and 1600-2500 kHz (circles in figure). Since it was shown in many studies that menadione can affect on calcium channels of cells [9] we also assume that the observed oscillations could be result of opening and closing of Ca^{2+} channels [14]. Evidence of this assumption is that existence of discovered oscillations is determined by types of ionic impurities contained in the sensor coating. Oscillations were not observed practically at all frequency range in experiments carried out with sensor coating which was formed on subphase contained Ce. Hence, atoms Ce can serve as blocker of calcium channels that agrees with data from [15, 16].

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(a) (b)

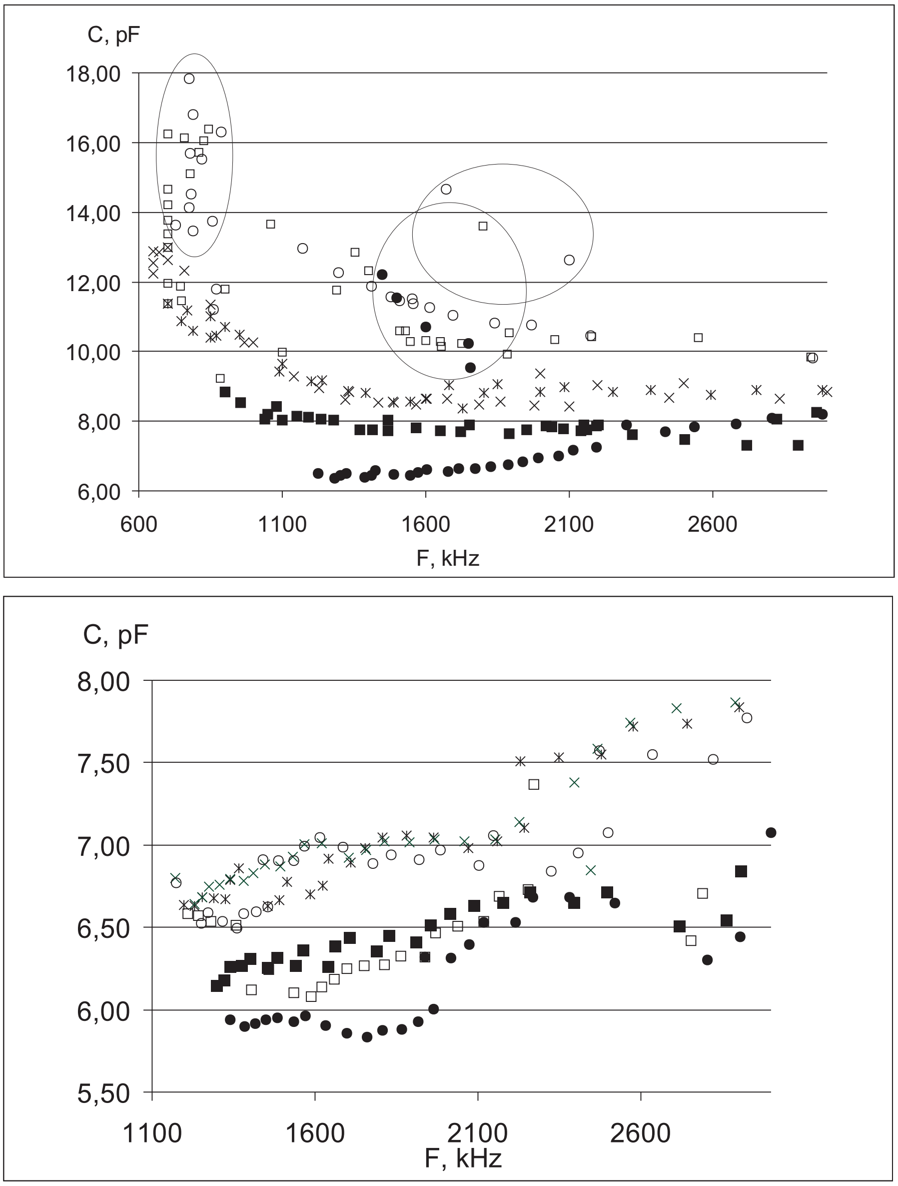

FIG. 4. Frequency branches of capacity oscillations (labeled in circles). A frequency dependence of the cell monolayer capacity was measured in a measuring chamber without menadione after menadione treatment during 30 min: - without menadione addition: fulfilled circles -direct branch, fulfilled squares - return branch, - at menadione addition: crosses - direct branch, asterisks - return branch, - the sensor with cell monolayer after menadione treatment during 30 min are in a measuring chamber without menadione: open circles -direct branch, open squares - return branch. In frequency branches of capacity the oscillations labels are the mean values of capacity.