Optically transparent electrodes to study living cells: a mini review

Artem Kuzovlev[1], Anatoly Evseev[2], Irina Goroncharovskaya[2], Aslan Shabanov[2], and Sergey Petrikov[2]

[1]Federal research and clinical center of intensive care medicine and rehabilitology
[2]N V Sklifosovsky Research Institute of Emergency Medicine

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Abstract

The use of electrochemical methods to study living systems, including cells, has been of interest to researchers for a long time. Thus, controlling the polarization of the electrode contacting living cells, one can influence, for example, their proliferation or the synthesis of specific proteins. Moreover, the electrochemical approach formed the basis of the biocompatibility improvement of the materials contacting with body tissues that use in carbon hemosorbents and implants development. It became possible to reach a fundamentally new level in the study of cell activity with the introduction of optically transparent electrodes in this area. The use of such materials allowed approaching to the study of the influence of the electrode potential on adhesion activity and morphology of the different cell types (HeLa cells, endothelial cell, etc.) more detailed. There are a negligible number of publications in this area despite the obvious advantages of the usage of optically transparent electrodes to study living cells. This mini review is devoted to some aspects of the interaction of living cells with conductive materials and current advances in the use of optically transparent electrodes for the study of living cells, as well as the prospects for their use in cellular technologies.

Abstract

The use of electrochemical methods to study living systems, including cells, has been of interest to researchers for a long time. Thus, controlling the polarization of the electrode contacting living cells, one can influence, for example, their proliferation or the synthesis of specific proteins. Moreover, the electrochemical approach formed the basis of the biocompatibility improvement of the materials contacting with body tissues that use in carbon hemosorbents and implants development. It became possible to reach a fundamentally new level in the study of cell activity with the introduction of optically transparent electrodes in this area. The use of such materials allowed approaching to the study of the influence of the electrode potential on adhesion activity and morphology of the different cell types (HeLa cells, endothelial cell, etc.) more detailed. There are a negligible number of publications in this area despite the obvious advantages of the usage of optically transparent electrodes to study living cells. This mini review is devoted to some aspects of the interaction of living cells with conductive materials and current advances in the use of optically transparent electrodes for the study of living cells, as well as the prospects for their use in cellular technologies.

Keywords: living cell, optically transparent electrode, indium tin oxide, polarization, morphology, cell adhesion

1. Introduction

Interest in using electrochemical methods to study living systems has been evident for decades. The simplicity, selectivity, sensitivity and relatively low cost of electrochemical methods allowed them to be widely

introduced in the field of medicine and biology. Moreover, their use is not limited only to analytical applications, but also underlies a number of medical procedures (electrochemical hemostasis, electrochemical lysis, iontophoresis, indirect electrochemical detoxification, application biocompatible coatings for endoprostheses and others (Sawyer, Srinivasan, Stanczewski, Ramasamy & Ramsey, 1974, Nilsson et al., 2000, Djokić, 2016, Gratieri, Santer & Kalia, 2017)).

2. Prerequisites of studies of the interaction of living cells with electrically conductive materials

Since the 50s of the last century, active work is being done to study the interaction of cells with foreign charged materials. For the first time, the assumption that the surface charge of an electrically conductive material affects the interaction of blood cells with this material was put forward when studying the mechanism of thrombogenesis. When blood contacted with metals having a positive potential value, a thrombus formed, while blood contacted with metals having negative potential values did not produce a blood clot. It was found *in vivo* that the value of the electrode potential of the order of +0.5 V (Ag/AgCl) was a critical value, after which there was an increased thrombus formation at the site of contact of blood and metal (Sawyer, Srinivasan, Stanczewski, Ramasamy & Ramsey, 1974). These data were consistent with *in vitro* experiments with external polarization, where it was shown that red blood cells and white blood cells were deposited on the surface of platinum and gold electrodes at a potential of +0.55 V (Ag/AgCl) (Saywer, Brattain & Boddy, 1964) and platelets were deposited at a potential of +0.65 V (Ag/AgCl) (Sawyer, Srinivasan, Stanczewski, Ramasamy & Ramsey, 1974). The phenomenon of cell deposition on the electrode was associated by the authors with the transfer of part of the charge from the cell membrane to the electrode surface, i.e. with the course of the electrochemical process. Red blood cell adhesion was also studied on a lead electrode in a solution of 0.012 M NaF at various electrode charge densities (Gingell & Fornes, 1976).

In connection with the development of technologies in tissue engineering, there is an increase in interest in research in this area, as evidenced by studies on the electrochemical adhesion and desorption of various types of cells (Jiang, Ferrigno, Mrksich & Whitesides, 2003, Inaba, Khademhosseini, Suzuki & Fukuda, 2009, Sun, Jiang & Jiang, 2011, Enomoto et al., 2016, Kobayashi et al., 2019).

Electrochemical ideas about the interaction of blood cells with a charged surface were used to study the traumatic ability of hemosorbents based on activated carbons in relation to blood cells (Goldin, Volkov, Goldfarb & Goldin Michael, 2006). Thus, the possibility of reducing the traumatic effect by external polarization of the hemosorbent was shown, and a potential region from -0.15 to +0.05 V (Ag/AgCl) was revealed, in which activated carbon remained indifferent to blood cells. The results formed the basis for the creation of composites based on activated carbon with an electrically conductive polymer deposited on its surface, which made it possible to create hemosorbents with desired properties without the need for external polarization (Khubutiya, Goldin, Stepanov, Kolesnikov & Kruglikov, 2012, Volfkovich, Goroncharovskaya, Evseev, Sosenkin & Goldin, 2017).

In addition, with the help of external polarization it is possible to regulate cell proliferation and cell synthesis of specific proteins. For example, under conditions of external polarization of the platinum electrode when the potential is shifted from +0.1 V (Ag/AgCl) to the region of more positive potentials, a decrease in the proliferation (division) of MKN45 line cells is observed, and at +0.4 V (Ag/AgCl) its complete inhibition (Kojima et al., 1991), and at the potential of about +0.4 V (Ag/AgCl), the maximum yield of carcinoembryonic antigen (CEA) synthesized by these cells is observed (Kojima et al., 1992).

Recently, studies of the electrochemical behavior of blood cells have resumed, which have experimentally proved the existence of electron transfer between cells and the electrode surface. Thus, it was found that erythrocytes undergo electrochemical reduction at a platinum electrode at potentials less than -0.15 V (Ag/AgCl), and electrochemical oxidation at potentials more positive than +0.2 V (Ag/AgCl) (Tsivadze et al., 2017).

The phenomena described above took place upon contact of cells with a foreign electrically conductive material under conditions of external polarization. At the same time, when studying a number of bacterial cells (for example, *E. Coli* , *Geobacter sulfurreducens* , etc.), the opposite effect of unidirectional transport

of electrons generated during the course of enzymatic processes from cell to electrode was discovered, which formed the basis for the development and creation microbial fuel cells (Bond & Lovley, 2003, Marsili et al., 2008, Scott & Yu, 2015).

3. Optically transparent electrodes

The introduction of optically transparent electrodes (Ellmer, 2012) used mainly in the production of liquid crystal displays and solar panels, has allowed reaching a qualitatively new level in studies of the interaction of living cells with electrically conductive materials. A wide range of materials can be used as optically transparent electrodes, from thin metal layers (for example, silver and copper widely used in the electronic industry (Bi et al., 2019) to the most innovative materials (for example, carbon nanotubes (López-Naranjo, González-Ortiz, Apátiga, Rivera-Muñoz & Manzano-Ramírez, 2016), electrically conductive polymers (3,4-ethylenedioxythiophene) (PEDOT) (Hofmann, Cloutet & Hadziioannou, 2018) and graphene oxide (Woo, 2019)) deposited on a transparent substrate. However, the most widely used are optically transparent electrodes based on indium and tin oxides (ITO) (Cao, Li, Chen & Xue, 2014, Afre, Sharma, Sharon & Sharo, 2018), which have a fairly high light transmission (80–95%) with a relatively low resistance (10–50 Ω/cm2) (Cao, Li, Chen & Xue, 2014).

4. The use of optically transparent electrodes for the study of living cells

Despite the obvious advantages when using optically transparent electrodes for the study of living cells, namely, the ability to visualize the behavior of cells in conditions of external polarization, this property was not realized in early works. In this case, either the effect of external polarization was studied without using the optical properties of the material, or the behavior of cells was visually examined, but without external polarization.

So, on the one hand, under conditions of external polarization of the ITO electrode without visual control, when studying mouse astrocyte cells, it was shown that at a potential of +0.3 V (Ag/AgCl) maximum secretion of neuron growth factor (NGF) is observed (Koyama, Haruyama, Kobatake & Aizawa, 1997) when studying thrombus formation, it was revealed that cathodic polarization prevented the formation of a thrombus (Schmitt, Baer, Meindl, Anderson & Mihm, 1984).

On the other hand, optically transparent electrodes can be used as the basis for creating cell patterns. For example, when a part of the ITO electrode surface is coated with gold modified to give it resistance to cells, the cells will selectively adhere to the ITO surface, which was controlled by the optical properties of the material (Jin, Yang, Zhang, Lin, Cui & Tang, 2009).

From our point of view, it is important to use the optical properties of materials to study their interaction with blood cells. Thus, in the absence of external polarization, it was shown that when red blood cells come in contact with materials such as glass coated with polydimethylsiloxane (-24 mV), glass coated with ITO (-39 mV) and glass without coating (-60 mV), the red blood cell morphology is shifted from discocytes to echinocytes as the surface potential shifts to more negative values (Mukhopadhyay, Ghosh, Sarkar & DasGupta, 2018).

From the electrochemical point of view, the phenomena of changes in cell morphology can be explained by changes in the transmembrane potential. For example, it is known that a change in the morphology of red blood cells, i.e. the transition stomatocyte - discocyte - echinocyte affects the change in the double electric layer at the erythrocyte membrane due to the influence of ionic strength and osmolarity of the environment (Bifano, Novak & Freedman, 1984, Glaser, 1993). Moreover, it was shown (Tachev, Danov & Kralchevsky, 2004) that this mechanism does not contradict the widespread bilayer pair theory (Sheets & Singer, 1974), where, as is believed, the band 3 protein plays the main role in the mechanism of changing the shape of the red blood cell (Betz, Bakowsky, Müller, Lehr & Bernhardt , 2007).

In connection with the foregoing, an obvious conclusion is drawn about the prospects of studying the interaction of living cells with optically transparent electrodes in conditions of external polarization. Schematically, the possibilities of using optically transparent electrodes for studying cells are shown in Figure 1.

The most detailed studies of the influence of the potential of an optically transparent electrode on cell morphology were performed using cultures such as HeLa cells, bovine aortic endothelial cells, *P. Fluorescens* , etc. (Yaoita, Shinohara, Aizawa, Hayakawa, Yamashita & Ikariyama, 1988, Wong, Langer & Ingber, 1994, Busalmen & de Sánchez, 2005).

When studying the effect of the ITO electrode potential on the morphology and growth of living HeLa cells at ITO potentials below +0.5 V (Ag/AgCl) no changes were observed, in the potential range from +0.5 (Ag/AgCl) to +0.7 V (Ag/AgCl) there is a reversible change in cell morphology and a decrease in cell proliferation rate, and at potentials above +0.7 V (Ag/AgCl) irreversible morphological changes begin, leading to cell death (Yaoita, Shinohara, Aizawa, Hayakawa, Yamashita & Ikariyama, 1988, Yaoita, Aizawa & Ikariyama, 1989, Yaoita, Ikariyama & Aizawa, 1990).

Also, using HeLa cells as an example, their adhesion and desorption from the surface of an ITO electrode depending on the applied potential was shown (Koyama, 2011). HeLa cells adhered to the ITO surface at +0.4 V (Ag/AgCl), while at -0.3 V (Ag/AgCl), the cells adhered to areas of glass not coated with ITO.

The behavior of bovine aortic endothelial cells on an ITO electrode coated with polypyrrole was studied by Wong, Langer & Ingber (1994). The authors note that on an oxidized polypyrrole film (without imposing potential), the cells attach to the surface and are flattened, at the same time, when the film is brought into a neutral state (-0.5 V), the cells attach, but remain round.

Studies on gold coated glass of *P. Fluorescens* culture (Busalmen & de Sánchez, 2005) showed that at a potential of -0.5 V (Ag/AgCl), bacteria do not adhere directly to the electrode surface, but form clusters, in contrast to the potential range from 0.1 In (Ag/AgCl) to -0.2 V (Ag/AgCl), where the cells showed exponential growth with a doubling time of 82.6 ± 7 min. At the same time, two different effects were detected in the anodic potential region. So, at +0.5 V (Ag/AgCl) an exponential growth is observed with a doubling time of 103 ± 8 min, while at +0.8 V (Ag/AgCl) the bacteria did not grow and a decrease in the area covered by bacteria was observed over time their entrainment from the surface with a solution.

In a study on an ITO electrode and an electrode based on gallium zinc oxide doped with yeast cells of *S. Cerevisiae* strain, it was shown that the cells attach to the electrode and show normal proliferation on the ITO electrode in the potential range from -0.2 V to -0.4 V (Ag/AgCl), while cell attachment to gallium doped zinc oxide did not occur (Koyama et al., 2015).

Regarding blood cells, as the most relevant object for research, it should be noted (Tsivadze et al., 2017, Goldin, Goroncharovskaya, Evseev, Shabanov, Goldin & Petrikov, 2019), where it was shown that the morphological state of red blood cells depends on the potential of the ITO electrode. In potentiodynamic conditions, i.e. with a linear sweep of the potential into the cathodic and anodic potential regions, the initial normal red blood cells (discocytes) were transformed into various morphological forms (echinocytes, spherocytes, stomatocytes) (Figure 2).

It is important that each of the morphological forms is formed and exists in certain ranges of potentials. When the ITO electrode potential shifts to the cathodic potential region at potentials less than -0.25 V (Ag/AgCl), transformation of discocytes into echinocytes was observed, which, when the potential shifts to even more negative values, transforms further into spheroechinocytes. When the ITO electrode potential shifted to the anodic potential region to a potential of +0.6 V (Ag/AgCl), no morphological changes were observed; more positively, the transformation of discocytes into stomatocytes took place. It is also important that the observed transformation of red blood cells was, as a rule, reversible. So, when changing the direction of scanning the potential, or turning off the current, a transition of the red blood cell to the initial state was observed.

The use of electrochemical impedance spectroscopy using optically transparent electrodes allows one to obtain additional information on the functioning of cells, for example, the synthesis of biologically active substances, the activity of ion channels, and adhesive activity (Choi, English, Jun, Kihm & Rack, 2007, Choi, English, Kihm & Margraves, 2007, Jahnke et al., 2009, Pänke, Weigel, Schmidt, Steude & Robitzki, 2011,

Jahnke, Schmidt, Frank, Weigel, Prönnecke & Robitzki, 2019).

5. Conclusions

The data presented in this mini review indicate the prospects of developments in the field of application of optically transparent electrodes for the study of living cells. Although questions remain about the nature of the observed phenomena in the blood cell / foreign charged surface system, the basis for answering these questions has already been laid. Obviously, the topic raised is highly relevant due to its interdisciplinarity, since it touches on a wide range of problems from identifying new fundamental aspects of the interaction of cells with charged surfaces to the application of optically transparent electrodes in cell engineering. In general, conducting research in this area using modern methods of analysis should not only solve fundamental problems, but also serve as a basis for the development of new diagnostic and therapeutic methods.

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Figure legends

Figure 1. Possibilities of using optically transparent electrodes to study living cells.

Figure 2. Morphological changes of red blood cells depending on ITO electrode potential (x400).