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Assessment of metabolic activity of human cells in solution and in polymer matrix with the use of metabolite-sensitive sensors

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ABSTRACT

We developed metabolite-sensitive electrochemical sensors on the basis of electrodes modified with a thick film of carbon nanotubes. Modified electrodes provide efficient pre-adsorption of cellular metabolites and their sensitive detection using anodic square-wave voltammetry. On the electrode surface both adhered and non-adhered human cells produce three oxidation peaks at the potentials of +0.82, +1.05, and +1.17 V attributed to three groups of cellular metabolites: amino acid-derived antioxidants including glutathione, guanine nucleotides, and also adenine nucleotides including ATP. The electrochemical response was well correlated with cell viability, intracellular ATP level and induction of apoptosis, as determined by independent assays. Developed sensors allow for robust and cost-effective assessment of ATP in cells in contrast to enzyme-based electrodes and conventional bioluminescent assay. Results can be used for rapid analysis of human cells for the purpose of medical diagnostics, transplantology, and toxicological screening. Additionally, we combined modified electrodes with human cells entrapped in agarose matrix. The resulting biosensor allowed for electrochemical monitoring of metabolic activity and death of cells within polymeric matrix that is of interest for tissue engineering applications.

1. Introduction

Biochemical analysis of mammalian cells is carried out in various biomedical studies [1,2]. Developmental and pathological processes in living cells are accompanied with a change in composition of plasma membrane components and intracellular metabolites [3]. Studying biochemical parameters of living cells is of particular interest in medical diagnostics of many human disorders [1] as well as for drug and toxicant screening [4].

Fluorescent probes/labeled antibodies are routinely used for the detection of individual components in fixed and live cell samples with the aid of optical microscopy [5]. Since flow cytometry has been introduced for quantitative fluorescent analysis it became an essential technique for identification and assessment of isolated cells [6,7].

Other modern techniques involve chromatographic separation of cell metabolites followed by their high-precision determination by means of mass spectrometry. Combined chromatography/mass spectrometry is an irreplaceable tool for metabolome research when informative analysis of metabolite profiles is required [1,8]. However, limitations of this tool, e.g. high cost, laboriousness, inapplicability to live cell assays, complicate its introduction to some biomedical applications including routine clinical diagnostics.

Cellular metabolites can be detected without separation by the use of spectroscopic techniques such as Raman spectroscopy. This technique allows for direct assessment of biochemical composition of living cells by Raman spectra under physiological conditions. Changes in Raman spectra provide valuable information about normal and pathological cellular processes [9,10]. However, Raman analysis of biological matter requires qualified interpretation of acquired data involving relatively complex mathematical methods.

During the last decade many efforts have been made to develop more handy tools for rapid assessment of key cellular metabolites. A promising platform for solving this problem is electrochemical sensors, especially voltammetric ones, which are characterized by selectivity, time efficiency and low cost of an analysis [11]. Electrochemical sensors can be used for direct detection of a wide range of biomolecules which are oxidized or reduced on the surface of electrodes. In certain cases the electrode is coupled with enzyme(s) to perform highly selective quantification of a metabolite of interest in biological sample [12]. To date, different electrochemical assays have been proposed for detection of catecholamines and nitric oxide on chemically modified electrodes [13–15] and also glutamate with the use of glutamate oxidase based sensors [16,17].

A reliable parameter of energetic status, viability and induction of apoptosis of human cells is intracellular ATP level [18–20]. Whereas some existing studies show the possibility of electrochemical detection of some unidentified metabolites in cells [21–23] they do not consider ATP and other adenine nucleotides which were shown to be oxidized with high overpotential at about +1.2 V [24]. For

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