Reagentless Microsensor Based on Conducting Poly(3-aminophenylboronic Acid) for Rapid Detection of Microorganisms in Aerosol


Abstract: We report on microsensor for direct detection of microorganisms in aerosol. Microsensor is based on interdigitated ultramicroelectrodes modified with electro-polymerized 3-aminophenylboronic acid (3-APBA). Appearance of Penicillium chrysogenum in aerosol as well as further increase of its concentration lead to poly(3-APBA) conductivity increase found by impedance spectroscopy. According to Raman spectroscopy data, the presence of microorganism affects polymer conductivity similarly to binding of glucose to polymer which results in self-doping phenomenon. The elaborated detection technique is 100 times faster comparing to agar plate cultivation: it requires less than 20 minutes for analysis. Dynamic range of microsensor includes the upper limit for non-contaminated air in Russian hygienic standard (500 colony-forming units per cubic meter). The first reported reagentless detection of Penicillium chrysogenum in aerosol by conductivity increase reveals a prospect for creation of a promising alternative to conventional techniques for detection of microorganisms.

Keywords: Electrochemical sensors · synthetic receptors · conductive polymers · boronic acid · Penicillium chrysogenum

Microbial contamination of indoor air is important for occupational and public health [1,2]. Among different microorganisms the microscopic fungi Penicillium, Aspergillus etc. are most common airborne genera [3,4]. They can cause mycosis, mycogenic food or drug allergies, rhinosinusitis and bronchial asthma [5–7]. Beside the harm to the human health there is also a negative influence of microorganisms on manufacturing processes of medicines, foods and microelectronics.

Currently, a few main techniques are applicable for microorganism detection in aerosol: agar plate cultivation, microscopic examination [8], DNA-related methods [9,10], ATP-bioluminescence [11], mass spectrometry [12] and flow cytometry [13]. All these methods suffer either from low specificity or require expensive equipment, special chemical reagents and time-consuming steps. In addition, it is usually impossible to apply them for continuous monitoring. This provides a significant interest in development of sensors offering simple, express and reliable detection methods. One of the most promising approaches for elaborating of sensors is based on biomimetics. Synthetic receptors, artificial antibodies made of aptamers, molecularly imprinted polymers recognizing certain interactions of functional groups possess a powerful combination of specificity of biorecognition elements and operational as well as storage stability.

Electrochemical techniques are among valuable analytical methods applicable for synthetic receptors since they can be performed with simple, miniaturized but sensitive equipment being independent of sample colour or turbidity and suitable for on-field measurements. One of the main challenges of electrochemical sensors is coupling electrochemical reactions with affinity interactions. This usually requires catalytic or electroactive label. However, including additional steps into analysis limits practical application of the sensors and makes impossible continuous monitoring. Therefore, reagentless sensors are particularly attractive providing non-destructive analysis of real objects. For development of reagentless electrochemical sensor it is possible to employ conducting polymer as a transducer for affinity interactions.

Phenylboronic acid based sensors are promising synthetic receptors recognizing 1,2- or 1,3-diol fragments [14]. These latter are common structural elements of saccharides and hydroxyacids incorporated within fungi cell wall [15]. Previously we elaborated novel reagentless detection principle based on affinity interactions of polyols (glucose, lactate, fructose etc.) with poly(3-aminophenylboronic acid) in aqueous solution [16]. Affinity interactions had first been shown to result in conductivity increase. This effect allows discriminating between specific and unspe-
APBA) due to specific interaction of the cell wall functional groups with boronic acid moiety which cause increase of conductivity of the polymer. Effect of poly(3-APBA) conductivity increase as a result of polymer self-doping in the presence of the fungi was confirmed by Raman spectroscopy. Detection of fungi with microsensor requires less than 20 minutes which is approximately 100 times faster than agar plate cultivation. Furthermore, microsensor is applicable to monitor hygienic standard of fungal content in air. Reagentless operation in air and compact size of microsensors opens the possibility to create embedded systems of air control in manufacturing processes and even in everyday life. The reported application of reagentless sensing principle is upcoming approach to detect complex analytical objects such as microorganisms in a simple manner opening new prospects for health care.

**Experimental**

Experiments were carried with Millipore Milli-Q deionized water (resistivity 18.2 MΩ cm at the room temperature). 3-aminophenylboric acid hydrochloride was purchased from Sigma-Aldrich (Germany), D-glucose and inorganic chemicals were obtained of the highest purity from Reakhim (Moscow, Russia).

Interdigitated gold ultramicroelectrodes with interelectrode distance of 10 μm were provided by Physics faculty of M.V. Lomonosov Moscow State University. Ultramicroelectrodes were pretreated in acidic solution the by applying cyclic voltammetric conditions in 0.05 M H₂SO₄ from 0 to 1.35 V with the scan rate of 40 mV s⁻¹ until stable cyclic voltammogram is obtained.

Cyclic voltammetry measurements were performed in a classical three-electrode cell. The working electrodes were: short-circuited interdigitated gold ultramicroelectrodes, carbon working electrode of three-electrode screen-printed planar structure (Rusens Ltd., Moscow, Russia). The counter electrode was a glassy carbon rod. All potentials were referenced to the system Ag/AgCl in 1 M KCl connected through Luggin capillary.

Scanning electron microscope LeoSUPRA 50 VP (Carl Zeiss, Germany) was used to observe ultramicroelectrode surface morphology and *Penicillium chrysogenum* suspension species imaging. Micrographs were recorded at 3.00 kV accelerating voltage and 3 mm working distance with InLens detector. Specimens with the microorganism were coated with 10 nm chromium films.

A Renishaw InVia Raman microscope (Renishaw, UK) acquired with 514 nm excitation laser source, 20× objective in the range from 1100 to 1700 cm⁻¹ was used for recording Raman spectra at the screen-printed electrodes modified with electropolymerized 3-aminophenylboronic acid.

Impedance spectra of polymer-modified interdigitated ultramicroelectrodes placed into aerosol flow were recorded at the room temperature using a Solartron 1255 frequency response analyser (Solartron, UK) with a homemade low-noise electrochemical interface. Measurements were made on the one of the two interdigitated ultramicroelectrodes in frequency range from 1 to 10000 Hz with sine-wave voltage amplitude of 0.005 V and direct current potential E_corr adjusted to 0 V versus the other one of the two ultramicroelectrodes.

Microscopic fungi *Penicillium chrysogenum* growing on a sterile solid agar medium in glass reservoir were provided by Department of Mycology and Algology of Biological faculty of M.V. Lomonosov Moscow State University. All microbiological experiments were performed in biological safety cabinet Laminar-S-1,8 (Lamsystems GmbH, Germany).

Microorganism concentration in each suspension was determined by counting colonies on agar plate on which a small portion of suspension was spread. For each suspension at least three agar plates were used and final concentration was calculated as a mean value. Aerosol for experiments with *Penicillium chrysogenum* was produced by passing air flow at the rate of 3 ml min⁻¹ through the glass flask filled with suspension. *Penicillium* concentration in aerosol was checked by counting colonies on agar plate on which a bioaerosol flow was directed. Particle distribution in aerosol with and without *P. chrysogenum* was obtained by aerosol particle detector AeroTRAK ((TSI Inc., USA) mounted near the outlet of aerosol flow from saturating suspension in the glass flask.

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**References**