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Microbial fuel cell-based diagnostic platform to reveal antibacterial effect of beta-lactam antibiotics☆

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ABSTRACT

Beta-lactam antibiotics comprise the largest group of antibacterial agents. Due to their bactericidal properties and limited toxicity to humans they are preferred in antimicrobial therapy. In most cases, therapy is empiric since susceptibility testing in diagnostic laboratories takes a relatively long time. This paper presents a novel platform that is based on the microbial fuel cell (MFC) technology and focuses on the early antibiogram determination of isolates against a series of beta-lactam antibiotics. An advantage of the system is that it can be integrated into traditional microbiological diagnostic laboratory procedures. Tested bacterium suspensions are uploaded into the anodic chambers of each miniaturized MFC unit integrated into a panel system, containing different antibiotic solutions. Electronic signals gained in each MFC unit are continuously monitored and are proportional to the metabolic activity of the presenting test bacterium. Using this method, antibiotic susceptibility can be evaluated in 2–4 h after inoculation. Hereby we demonstrate the efficacy of the platform in antibiogram determination by testing the susceptibilities of *Escherichia coli* strain ATCC 25922 and *Staphylococcus aureus* strain ATCC 29213 against 10 beta-lactam antibiotics (penicillin, ampicillin, ticarcillin, cefazolin, cefuroxime, cefoperazone, cefepime, cefoxitin, cefaclor, imipenem). This paper also presents the construction of the background instrumentation and the panel system into which a printed circuit board (PCB) based electrode was integrated. Our results suggest that MFC based biosensors have the potential to be used in diagnostics for antibiogram determination.

1. Introduction

The spread of antibiotic resistance is one of the most significant health care issues or our times [1]. This process is chiefly generated by inadequate antibiotic prescription habits. In most cases empiric antibiotic therapies are used due to the fact that clinicians do not wait for results from microbiological diagnostic laboratories. If time-consuming specimen processing could be reduced to a manageable level (a couple of hours), a targeted therapy could be initiated earlier, which could eventually limit, the presently used empiric therapy.

At present, the cheap and easily interpretable Kirby-Bauer disk diffusion method is used by most laboratories for revealing the susceptibility of the tested infectious agent [2,3]. The advantage of this traditional growth-based method is that it is phenotypic nevertheless, it takes a relatively long time (24–48 h) till the result becomes available to the clinician. Molecular based methods, such as simple and multiplex PCR, DNA sequencing, macro- and microarray are also applied in well-equipped laboratories in order to routinely detect the presence of certain resistance genes [4]. Presence of the detected gene, however, does not necessarily mean that it is also functional, as demonstrated earlier [5,6] in the case of beta-lactams which comprise the largest group of antibacterial agents, with dozens of derivatives available for clinical use [7]. The popularity of these agents results from their bactericidal action and lack of toxicity. These drugs target and inhibit cell wall biosynthesis and since most clinically relevant bacteria have cell walls, beta-lactam agents act against a broad spectrum of gram-positive

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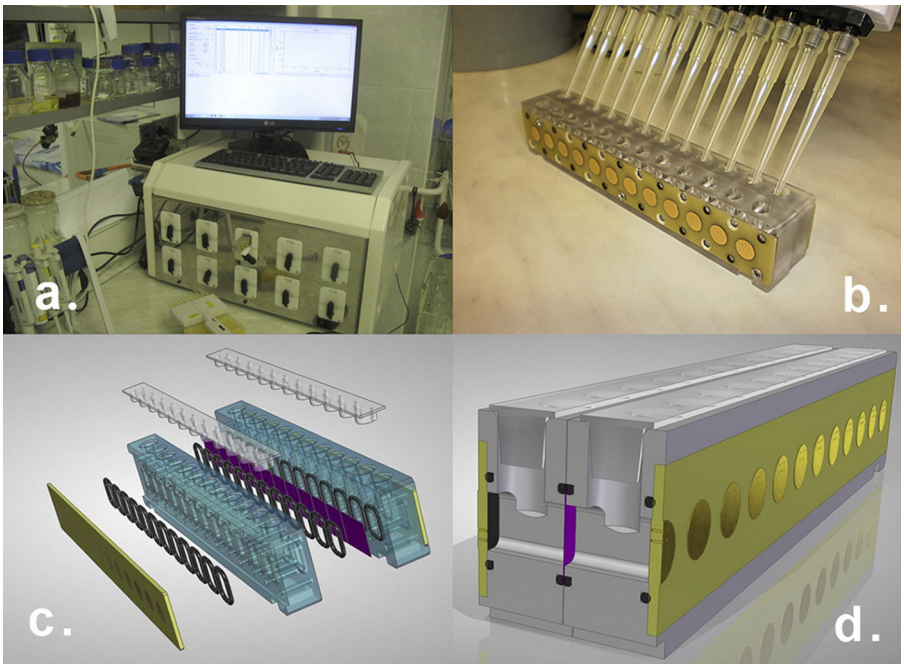

Fig. 1. Illustration the buildup of the MFC-based biosensor platform. The platform (a) used in this study is based on a panel system in which upload of bacterial samples can be carried out by using a simple multichannel pipette (b). During assembly, each part of the panel system is sandwiched (c). Anodes and cathodes of the μl-scale MFCs are integrated into a PCB base, which also underlie the sides of the panel and involve the outer contact points for the instrument (d).

and gram-negative bacteria [7]. Many factors (species of bacteria, type and number of penicillin binding proteins, structural characteristics, resistance mechanisms, etc.), however, contribute to a beta-lactam's spectrum, and for this reason laboratory testing should anticipate therapy in order to choose the best drug of choice. It is of vital importance since the clinician has to know whether the infection-causing agent in question is susceptible to a certain antibiotic or not. To identify this, a fast phenotypic method is needed.

Microbial fuel cells (MFCs) are electrochemical devices in which metabolic activities of the tested microorganisms can be monitored. If microbes in the anodic chamber are physiologically active, they will establish a potential difference between the anode and the cathode assuring a driving force for an electron flow. MFCs are thought to be potent devices for harvesting the energy from wastes and for this reason they can assure sustainable energy production. Only recently have they been considered as potential biosensors [8–10], where microorganisms in the anode compartment act as biocatalysts and the electrodes and proton exchange membrane serve as transducers. The signal is gained directly as a measurable electric signal. In comparison with other existing sensors (i.e. pH, temperature), MFC biosensors work as small bioreactors with high selectivity [11]. Miniaturization is critical for the development of MFCs for biosensor applications. Not only miniaturization but also the establishment of an efficient electron transfer between the bacterium and the anode is a key factor for proper functioning. This can be facilitated by different methods [12,13].

In this paper we demonstrate the applicability of an MFC-based biosensor for fast determination of the susceptibility of two tested pathogenic bacteria against 10 different beta-lactam antimicrobial agents belonging to different classes and subclasses. Although, miniaturized two-chamber MFC systems in μl scale range have been reported earlier [14–16], none of them applied the printed circuit board (PCB) technology for establishing a graphite electrode system. Since this technology is ideal for large scale electrode production it can promote the spread of MFC based biosensor applications in the future.

The advantage of the system in the determination of the antibiotic susceptibility testing is that it is (1) phenotypic, (2) results can be interpreted within 1–4 h after inoculation, (3) susceptibility of an infectious agent against several antibiotics can be determined parallel. These could result in an earlier targeted antibiotic therapy and reduce unnecessary antibiotic usage that is not only a waste of money, but also contributes to the spread of antibiotic resistance among bacteria.

2. Materials and methods

2.1. Platform design, production and assembly

The panel and the complete platform (Fig. 1) were designed by the 3D modeling software Solid Edge ST5 (Siemens PLM). Compartment bodies of the anodic and cathode chambers were microfabricated of plexi on the CNC routers TMV850 and EMR610. Between the two chambers activated Nafion N115 was applied as proton selective membrane and external sides of the chambers were covered by PCB (type FR4) containing the integrated electrodes (Fig. 1c and d). These special PCB-based sides, that did not only contain the electrodes but also assured connection between the chambers and the instrumental part of the platform, were designed by the Expedition Enterprise PCB design software (Mentor Graphics, USA). Production was carried out according to the resulting Gerber file. The PCB was manufactured with the standard PCB manufacturing process. FR4 (woven fiberglass cloth with an epoxy resin) was applied as a base material for the board and copper thickness was 17.5 μm on both sides. Instrumental side of the copper surface was coated with ENIG (electro less nickel immersion gold), while inner sides (anodic and cathode chamber side) with graphite (Fig. 3a). Between the two sides true hole VIAs assured electric connection (information about the manufacturing process: http://www.eurocircuits.com/).

After production each part of the platform-except the Nafion membrane-containing the miniaturized MFC units were washed in acetone for 15 min, in sterile distilled water for three times, once in 96% ethanol for 15 min, air dried at 75 °C for 15 min and assembled by sandwiching them (Fig. 1c and d) under a laminar flow box to assure germfree conditions. After construction, cathode chambers were filled up with 0.02 M potassium hexacyanoferrate (III) solution, while the anodic chambers with 200 μl of 0.1 M phosphate puffer-based medium containing the proper antibiotic with more than two-fold higher concentration than the