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Nanochannels for electrical biosensing

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ABSTRACT

This review shows the recent trends on the use of both single and array nanochannels for electrical biosensing applications. Some general considerations on the principles of the stochastic sensing, together with an overview about the common routes for nanochannels preparation before focusing on the applications for DNA, protein, virus, toxin and other analytes detection are given. Emerging materials used to obtain nanochannels, such as graphene and its analogues as well as novel systems based on the use of nanoparticles in combination with nanochannels are discussed. Aspects related to the analytical performance of the developed devices are also discussed. Finally prospects for future improvements and applications of this technology are included.

Contents

1. Introduction

One of the leading sectors of state-of-the-art nanoscience and nanotechnology is focused on the development and application of novel biosensing systems. Measurement of clinical parameters, monitoring of environmental pollutants, detection of pathogens, and other industrial and safety and security related detection are reported in

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the wide range of biosensing systems that are under commercial development [1,2].

One of the challenges for providing biosensing devices to end-users relies in the modification of current technologies so as to make them run on everyday equipment, rather than on specialized apparatuses. Recent advances in nanomaterials and nanotechnologies research are allowing the development of either highly improved biosensing systems or brand new ones fulfilling the requirements of cost-effective and user-friendly devices [3–5].

Particularly, nanopores/nanochannels have emerged in the last decade as materials with powerful analytic capability for biosensing applications. Natural protein-based ion channels have inspired artificial electrical biosensing systems based on both single nanochannels and nanochannel arrays with outstanding performance for the detection of a variety of analytes, including protein biomarkers and DNA between others. The potential ability of these devices for DNA sequencing are one of the most exciting perspectives of the nowadays nanobiotechnology.

Electrical sensing using single nanopores have been object of several reviews [6–14]. In addition biosensing applications of nanochannel arrays, but mostly related to optical approaches which has drawbacks in terms of application in real samples beside inappropriateness of the detection principle for in-field /point of care applications are also found in the literature [15,16]. In contrast, in this review we focus for the first time in a more analytical application-related perspective on single nanochannel-based electrical sensing platforms, giving a special relevance to the emerging materials used for drilling single nanochannels as alternative to the traditional silicon oxide/silicon nitride membranes, such as graphene and its analogues (hafnium oxide, boron nitride and molybdenum disulphide). We also highlight the recent trends in the development of innovative electrical approaches based on nanochannel arrays, in combination with the use of nanoparticles.

For a better understanding of this review, it is important to clarify first that "nanopore" is defined as a pore with a diameter in the range 1–100 nm, and a depth shorter than its diameter. The term "nanochannel" applies when the pore depth is much larger than the diameter which makes them more selective on the translocated analytes [17]. In spite of these differences, both terms are currently in common mutual use.

2. Basic concepts: from the coulter counter to the stochastic sensing

The precursor of the nanochannels-based biosensing systems was the microparticle counter device developed by Wallace Coulter in the 50's [18–20]. Mobility, surface charge, and concentration of micro-sized analytes can be evaluated by simply measuring changes in the electrical conductance (electric current or voltage pulse) between two chambers separated by a microchannel when such analyte passes through the channel. Commercial devices based on such principle are routinely used in the industry of painting and ceramics as well as in hospitals (for cells counting/determination) between other applications (Fig. 1A).

This device evolved from micro- to nanochannels-based ones, shown to be able to detect molecules in the nanoscale such as ssDNA and proteins (Fig. 1B). However, the preparation of two chambers separated by a thin membrane containing a single pore with nanometer size represents a major technical challenge. As in many cases, nature has served here as inspiration, providing with alternatives such as the use of pore forming toxins (PFTs) [22]. PFTs are protein exotosins typically produced by bacteria such as *Clostridium septicum* o *Staphilococus aureus*. They are the most common class of bacterial protein toxins and the responsible of the most important virulence factors. These toxins are secreted by bacteria in a water-soluble form. Their monomers diffuse towards the target cells, bind them via specific receptors and oligomerize forming ring-like complexes. The pore formation leads to ionic imbalances in the cells and the subsequent cell death (in the case of erythrocytes, to cell lysis). In some cases PFTs also transport secondary toxins into the cells via the pores, so the toxic effect is even higher.

As example of PFTs we can find i.e. α-hemolysin, anthrax toxin or pneumolysin, between others. In both cases, they form heptamers which are the responsible of the pore formation. The structures of the pores formed by α -hemolysin and the anthrax toxin are shown in Fig. 1C while the mechanism of action of these toxins is illustrated in Fig. 1D.

The insertion of the α-hemolysin bacterial protein pore in artificial lipid bilayer membranes separating two chambers filled with an electrolyte solution was the pioneer approach to build these biomimetic nanochannels [21,23,24]. A drop in electric potential close to the nanochannel is created when an external electrical field is applied across the membrane, producing an ionic flow and inducing a measurable current. Charged biopolymers (i.e. DNA) are first attracted by the electric field in a random way and then forced to pass through the channel. This pass, also known as "translocation" produces a displacement of electrolytes in the channel, leading to a sudden decrease in conductivity which can be recorded with an electrometer.

The ability to use a single channel as an analytical sensor, was reported for the first time in the early 1990s by Kasianowicz and Bezrukov who demonstrated the ability of such systems to detect and discriminate between different ions [25,26]. These findings opened the way of extensive sensing applications based on the so-called stochastic sensing, being the work of Bayley and co-workers considered as reference in this area [27]. The selectivity of the system is given by specific receptors that can be inserted inside the nanochannel by genetic engineering techniques, allowing to detect a variety of analytes such as DNA, proteins and others, being the possibilities for DNA sequencing thoroughly studied [28,29] as will be detailed in the following sections.

Solid-state nanochannels have emerged in the last years as alternative systems able to solve the drawbacks inherent to the biological ones, in terms of better stability, lower time of analysis, easier functionalization and precise pore-size control, representing reliable robust sensing platforms [11,12,30]. Such solid-state nanochannels can be prepared following different methodologies, as will be detailed later. Some of these methodologies also allow to obtain nanochannel arrays, which have led to novel sensing systems both electrochemical [16] and optical [31–34]. In this review we will focus only on the electrochemical approaches, as summarized at section 4.

In the following sections an overview of the most representative and recent works related to the single-nanochannel resistive biosensing and the novel approaches developed for protein and DNA sensing using nanochannel arrays will be given. Electrical systems using single nanochannels are mostly based on the stochastic sensing, so they will be classified in this review in relation to the detected analyte. Nanochannel arrays-based sensing systems will be classified regarding the electrical technique used for the analyte detection.

3. Electrical biosensing systems based on single nanochannels

3.1. Single nanochannel preparation

As stated above, the most widely used biological nanochannel for biosensing is the heptameric α-hemolysin bacterial protein pore from *Staphilococus aureus* which consists of a transmembrane β-barrel and a big cap region sticking out [35]. The nanochannel diameter in the entrances ranges from 2.9 nm (cis entrance) to 2 nm (trans entrance) while the internal cavity and the inner