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Reusable conductimetric array of interdigitated microelectrodes for the readout of low-density microarrays

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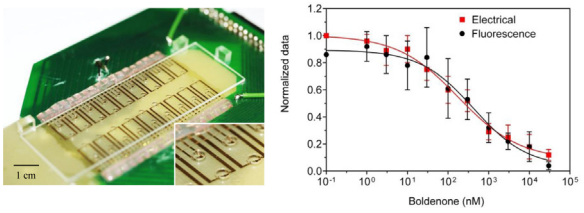
HIGHLIGHTS

- Array of interdigitated electrodes for carrying out up to thirty-six simultaneous analyses.
- Application to the readout of protein microarrays developed on disposable glass slides.
- Compact and inexpensive instrumentation is just required.
- Analytical performance comparable to fluorescent microarray counterparts.
- Detection of boldenone achieving limits of detection below the threshold value set by WADA and EU.

GRAPHICAL ABSTRACT

Reusable array of interdigitated electrodes applied to the electrical readout of a protein microarray for boldenone steroid hormone developed on a disposable glass slide.



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ABSTRACT

Low-density protein microarrays are emerging tools in diagnostics whose deployment could be primarily limited by the cost of fluorescence detection schemes. This paper describes an electrical readout system of microarrays comprising an array of gold interdigitated microelectrodes and an array of polydimethylsiloxane microwells, which enabled multiplexed detection of up to thirty six biological events on the same substrate. Similarly to fluorescent readout counterparts, the microarray can be developed on disposable glass slide substrates. However, unlike them, the presented approach is compact and requires a simple and inexpensive instrumentation. The system makes use of urease labeled affinity reagents for developing the microarrays and is based on detection of conductivity changes taking place when ionic species are generated in solution due to the catalytic hydrolysis of urea. The use of a polydimethylsiloxane microwell array facilitates the positioning of the measurement solution on every spot of the microarray. Also, it ensures the liquid tightness and isolation from the surrounding ones during the microarray readout process, thereby avoiding evaporation and chemical cross-talk effects that were shown to affect the sensitivity and reliability of the system. The performance of the system is demonstrated by carrying out the readout of a microarray for boldenone anabolic androgenic steroid hormone. Analytical results are comparable to those obtained by fluorescent scanner detection approaches. The estimated detection limit is 4.0 ng mL^{-1}, this being below the threshold value set by the World Anti-Doping Agency and the European Community.

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1. Introduction

Research into the development of analytical tools that enable multiplexed detection for diagnostics is growing exponentially [1,2]. The highly parallel and high-throughput nature of microarrays makes them ideally suited for monitoring genetic alterations as well as protein expression processes directly related to a variety of diseases [3–5]. The potential of these devices for point-of-care diagnostics to be applied in decentralized screening programmes and as analytical tools in a personalized medicine scenario is huge considering the minute amounts of sample and reagent volumes required together with the low-cost of the common glass-like slide substrates applied to carry out the corresponding affinity reactions [6].

Readout of microarrays is routinely carried out using optical methods [7–10]. Among them, fluorescence and, to less extent, chemiluminescence scanning approaches have been frequently chosen aiming at achieving the required sensitivity for the detection of a target analyte. However, both methods require bulky and expensive benchtop equipment. Nevertheless, other detection schemes can be applied that make use of compact and cost-effective instruments without compromising the required sensitivity and specificity of this kind of analyses. To this end, electrical readout systems are more and more being introduced in multiplexed/multiparametric-based systems [11–15]. They usually consist of arrays of individually addressable electrodes with the ability to transduce the selective affinity interaction for a specific target analyte on a single spot of the microarray into an electrical signal. These systems featured high signal-to-noise ratios, low number of components and low power requirements, which result in compact and inexpensive instruments, amenable to be portable [16]. Examples of electrical systems already on the market are the ElectraSense™ Reader from CombiMatrix [17,18] or the eSensor detection technology developed by GenMark Diagnostics, Inc. [19,20] for DNA high-density and low-density microarrays, respectively. Both of them are based on the amperometric measurement of a faradaic current produced by the redox process of an electroactive molecule used as a label or generated by an oxidoreductase enzyme label. Other reported approaches rely on different transduction modes such as potentiometry or electrochemical impedance spectroscopy and incorporate arrays of field effect transistors [21–23] or interdigitated electrodes (IDEs), respectively [14,24]. However, all of them have in common the immobilization of the specific bioreceptors on the electrode surface. This implies that the microarrays are developed on special substrates containing the required electrodes and in turn, specific tailor-made strategies for the immobilization of the biologicals are required. In this context, and taking into account that the microarrays are mostly single-use, the current technology makes the production of electrical microarray systems to be not so cost-effective.

By combining the merits of fluorescent scanner and previously reported electrical readout approaches, the proof-of-principle of an electrical readout system of microarrays, comprising an array of gold IDEs, which enabled multiplexed impedance detection of up to 36 biological events on the same substrate, was previously reported by our group [25]. Similarly to optical readout counterparts, regular glass slides were applied for carrying out the microarray. However, an electrical readout approach was implemented, which was based on monitoring solution conductance changes by applying urease-based (urease labeled) immunoassay reactions. The hydrolysis of urea catalyzed by urease produces ionic species in solution and increases its conductivity [26]. This change could be directly related to the target analyte concentration being measured [27]. Following the completion of the required affinity reactions of a microarray on a slide, this was placed over the IDE array leaving a 300 μm-high gap and aligned so that each spot faced one IDE. 1.7 μL-droplets of urea solution were placed in the gap to contact the IDEs and the corresponding spots of the microarray. Then, the analytical signal was recorded over a time period at a set frequency at which the changes in the solution conductance were unambiguously measured.

The developed electrical readout was affected by droplet evaporation and chemical cross-talk between adjacent droplets during the readout of a microarray. Also, it required the use of a microdispenser to accurately position the droplets on the electrodes. In this work, the second generation of the system is described in detail. An array of elastomeric microwells was integrated in the system, which enabled first, to easily place the required urea solution on the IDE array without using any specific instrumentation and second, to ensure the liquid tightness on each spot once the microarray was positioned over it.

Polymeric microwells were fabricated by soft lithography using polydimethylsiloxane elastomer (PDMS). Since the pioneering work of Whitesides almost two decades ago [28], a wide range of different soft lithographic techniques have been developed. Those ones based on molding processes from a master have been applied to the fabrication of uncountable number of micro- and nanostructures, many of them being implemented in the development of lab-on-chip analytical devices [29,30]. The design and fabrication of PDMS microwells showing different geometries together with other components was carried out for the development of automatic immunoassays and cell culture purposes. Among the former, a microwell-patterned assay chamber was integrated in a microfluidic chip and applied to the immunoenzymatic detection of low abundant proteins [31]. The latter include the fabrication of rounded bottom PDMS microwell arrays for the study of single cell enzyme kinetic analysis [32] and a microfluidic system containing a 16-microwell array with the capability of simultaneously carrying out cell culture and drug testing under various oxygen tensions [33].

The PDMS microwell array described in this work, when aligned on top of the IDE array, provides the electrical readout system with the required features to make it more user-friendly and to enhance its sensitivity. The analytical potential of the system is demonstrated by carrying out the electrical readout of a microarray for the detection of boldenone [34]. Boldenone is an anabolic androgenic steroid (AAS). AASs are used illegally to improve athletic performance in sports and to increase meat production in the agro-alimentary field. AASs are completely prohibited by the World Anti-Doping Agency (WADA) [35] and the European Community [36].

2. Experimental

2.1. Materials

Ammonium bicarbonate, ammonium hydroxide, glycine, urease from Jack Bean (Type III, 15,000–50,000 units g^{-1} solid), urea, phosphate buffered saline (PBS) tablets, Tween 20, 3-glycidoxypropylmethyldiethoxysilane (GPTMS) and streptavidin-Cy3 were purchased from Sigma–Aldrich (St. Louis, MO, USA). Boldenone was purchased from Sequoia Research Products, Ltd. (Oxford, UK). Glass slides were obtained from Corning (Pozuelo de Alarcon, Spain) and functionalized with GPTMS. Haptenized BSA (13BSA) and biotinylated antibody (anti-boldenone, As138-B) were prepared in the laboratory of the NB4D group and previously tested for the detection of boldenone in ELISA plates [34]. The Lightning-Link Streptavidin conjugation kit (Innova Biosciences Ltd.) was purchased from Antibody BCN (Barcelona, Spain) and used to produce a streptavidin–urease conjugate. All other chemicals were of analytical reagent grade and used as received.

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