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Development and impedimetric evaluation of a magnetic interdigitated microelectrode

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ABSTRACT

A magnetic interdigitated microelectrode (m-IDμE) has been developed, characterized and evaluated. In order to demonstrate the potential use of these electrodes in the biosensors field, impedimetric measurements of bovine serum albumin (BSA) biofunctionalized magnetic μ-particles (*BSA*MP) were performed. Thanks to their magnetic capabilities and to the use of magnetic μ-particles, the developed electrodes were successfully regenerated and could be reused several times. The classical stepped-sine impedance spectroscopy (IS) technique and the state-of-art frequency response analyzer (FRA) multisine IS method based on the local polynomial method (LPM) were used as measurement techniques. The significant reduction of measuring time and accuracy of the multisine IS LPM-based technique reveals a promising performance for fast and accurate real-time on-line screening applications.

1. Introduction

Over the past years, chemical analysis has been revolutionized by the biosensors technology [1,2]. Causes for this great advance are the many advantages and possibilities that biosensors offer, such as selectivity, sensitivity, accuracy, portability, miniaturization and fast response, among others.

To capture the biological recognition element, e.g. antibody or antigen, self-assembled monolayers (SAM) of organic molecules are widely used to functionalize the electrode surface [3–6]. Although the regeneration of these electrodes has been reported, the use of chemicals, e.g. HCl, glycine–NaOH or glycine–HCl, is necessary. Still, the regeneration process is not a straightforward task since there is a risk of losing sensitivity and/or leaving biological samples on the surface [7].

As the use of disposable electrodes implies a high cost and the regeneration process (1) involves the consumption of chemical and (2) is time consuming, the combination of interdigitated electrodes (IDEs) and magnetic capture could be a cost-effective alternative due to its possibility of being reusable. By means of this approach, the magnetic μ-particles (MP) are used as a solid substrate for the biological elements (e.g. antigen, antibody). Thus, these biological elements are first, covalently attached on the surface of MP and then magnetically attached on the surface of the electrode by including a magnet on the bottom of the electrode. Then, the electrode is free of any biological residue when the magnet is removed followed by simple cleaning with ultra pure water.

The use of impedimetric biosensors based on magnetic capture on the surface of electrodes has been recently described in the literature, for example for screen-printed electrodes [8,9] or gold electrodes [10]. In addition, in the last 10 years some papers

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studying magnetic impedance with a focus on biosensing have appeared [11–14]. However, little has been said about the application of magnetic capture applied to impedimetric biosensors using interdigitated microelectrodes (IDμE) [15,16], a technology that deserves further study and refinement, especially taking into account the advantages that IDμEs offer instead of conventional electrodes such as: (1) improvement in the sensitivity thanks to the micro-miniaturization, providing a small dielectric gap between the electrodes [17] and also a large aspect ratio (width/length) of the electrodes; (2) reproducible fabrication and potentially very low cost for mass production; (3) direct immunosensing, avoiding labels (label-free biosensors), for example, radioactive, fluorescent, enzymatic or electrochemical [18].

Regarding the techniques applied to impedimetric studies [19], those of them in which the measurement time is not relevant have been based on classical digital/analog stepped-sine based impedance analyzers based on using an auto-balancing bridge, e.g. HP4192 [20]. However, the measuring instrument that has revolutionized the IS measurements over the past decades is the frequency response analyzer (FRA). First FRA devices, e.g. Solartron 1174, determined the impedance by correlating, at each frequency, the response with a phase and quadrature signals and integrating [21,22]. Despite being a relatively simple and effective method, the measurement time may be too large for some applications, for example, for high-throughput m-IDμE monitoring. According to [23], for a logarithmic analysis carried out between 0.01 and 10 Hz with five frequencies per decade, Solartron 1174 needs 276 s including the calculation time. If a linear sweep is considered, then 1566 s are necessary (i.e. 1245 s for the acquisition time and 321 s for the total calculation time). The first impedance frequency response measurement without frequency scanning goes back to 1946 with the use of binary signals [24]. Later in the 1970s, the use of multisines was introduced [25–27]. To date, multisine signals still continue to be used in many studies because it retains the advantages of a sine wave while reducing the measuring time [28–31]. Recent advances in digital signal processing, i.e. the Local Polynomial Method (LPM) [32], have opened up a new era for multisine-based FRA-IS instrumentation in these applications where measurement time matters.

This article aims to contribute to the understanding of the impedimetric response of magnetic interdigitated microelectrodes (m-IDμE). To do that, we first evaluate if the IDμE developed in this work can be used as a reusable biosensor based on MP using bovine serum albumin (BSA) biofunctionalized MP (*BSA*MP) as analyte. Then, we compare the multisine IS LPM-based approach w.r.t. the conventional stepped-sine spectroscopy method considering five concentrations of *BSA*MP in the range of 0–320 μg mL^{-1} for obtaining a rapid and accurate biosensor characterization. Finally, we determine the ability of the system to detect the presence of molecules, e.g. proteins like antibodies, on the surface of the MP.

2. Materials

2.1. Device fabrication

Sensing devices are IDμE on top of a glass chip. Each chip module contains six electrodes and measures approximately 23 mm × 19 mm in total, as depicted in Fig. 1(right). The fabrication process uses standard microelectronic fabrication methods. The geometry used for the devices is shown schematically in Fig. 1(left). Each device consists of two weaved comb-like gold electrodes emerging from wide busbars. Each comb finger is about 5 μm width with a near 5 μm gap. The area covered by the fingers is about 3.4 mm × 3.4 mm.

The fabrication method used to make the devices is shown in the lower panel of Fig. 1(left). The lift-off technique was used to obtain such small features with good yield. Other methods like metal wet etching resulted in poor yield with inconsistent metal coverage and many unusable devices. For the lift-off fabrication process, a Schott glass wafer is prepared by standard cleaning procedures and afterwards spin-coated with photoresist. Starting from a wafer coated with photoresist, a mask pattern of the IDμE is then transferred to the resist by photolithography (Fig. 1(a)), and etched exposing the wafer (Fig. 1(b)). The exposed areas are subsequently removed after developing. The wafer is then rinsed in deionized water and dried. Once the mask is defined, first thin layer of about 10 nm of chromium is deposited by RF sputtering (Fig. 1(c)). This step is necessary to improve adherence to the glass surface as gold has very low adherence to glass and easily peels off. After the Cr is deposited a thick Au layer near 300 nm is sputtered to form the complete electrodes (Fig. 1(d)). The sputtering process covers the full wafer with metal, but after photoresist release, the excess metal on top of the unexposed resist, is finally removed. Next, the wafer is cut in 23 mm by 19 mm dices. Finally, the IDμE were characterized by profilometry measurements and cyclic voltammetries measurements (see Supplementary data, Fig. 4). Finally, a neodymium magnet array was placed on the bottom of the IDμEs in order to obtain the magnetic properties of the m-IDμEs.

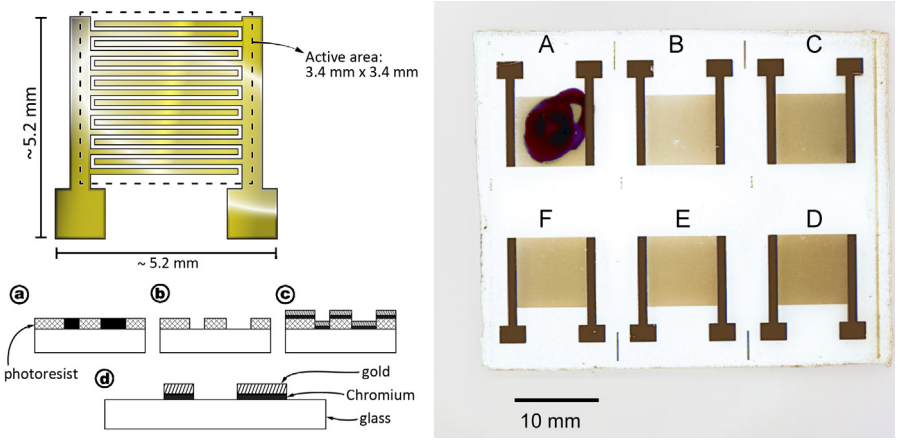

Fig. 1. (Left) Schematic representation of the interdigitated μ-electrodes (IDμE) structure and fabrication process flow. From the top view, each device measures approximately 5.2 mm × 5.2 mm, having an active area (where the fingers of each electrode weave) of about 3.4 mm × 3.4 mm; (right) chip module showing the sensing devices. Each chip consists of six IDμE A to F. For identifying purposes, the first device A is marked with a dot on the backside of the glass wafer.