



Design and characterisation of a thin-film electrode array with shared reference/counter electrodes for electrochemical detection



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ABSTRACT

In the current study, a novel electrode array and integrated microfluidics have been designed and characterised in order to create a sensor chip which is not only easy, rapid and cheaper to produce but also have a smaller imprint and good electrochemical sensing properties. The current study includes the assessment of the effects of an Au quasi-reference electrode and the use of shared reference/counter electrodes for the array, in order to obtain a small array that can be produced using a fine metal mask. In the study, it is found that when Au is used as the quasi-reference electrode, the arrays with shared reference and counter electrodes result in faster electron transfer kinetics and prevent the potential change with respect to scan rate, and hence is advantageous with respect to conventional electrodes. In addition, the resulting novel electrode array has been shown to result in higher current density ($10.52 \mu\text{A}/\text{cm}^2$; HRP detection assay) and measured diffusion coefficient ($14.40 \times 10^{-12} \text{cm}^2/\text{s}$; calculated from the data of cyclic voltammetry with 1 mM potassium ferricyanide) with respect to conventional electrodes tested in the study. Using the new electrode arrays, the detection limits obtained from horse radish peroxidase (HRP) and bisphenol A assays were 12.5 ng/ml ($2.84 \times 10^{-10} \text{M}$) and 10 ng/ml ($44 \times 10^{-9} \text{M}$), respectively. Performing the HRP detection assay in a flow injection system using array integrated microfluidics provided 25 times lower detection limit ($11.36 \times 10^{-12} \text{M}$), although Ti has been used as electrode material instead of Au. In short, incorporation of this new electrode array to lab-on-a-chip or MEMs (micro-electro mechanic systems) technologies may pave the way for easy to use automated biosensing devices that could be used for a variety of applications from diagnostics to environmental monitoring, and studies will continue to move forward in this direction.

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1. Introduction

Biosensors have been envisioned to play a significant analytical role in diagnostics, bioprocesses, quality assurance in agriculture and food industries, environmental monitoring and homeland security. All of the mentioned sectors have considerable market size. One example is the global food safety testing market by contaminants which is estimated to grow at an annual growth rate of 10.46% to \$2.5 billion in 2015 (MarketPublishers, 2011). Another important application area is in vitro diagnostics (IVD) with a yearly worldwide market of \$42 billion in 2007 (KaloramalInformation, 2008). Therefore, further research to develop new biosensor devices for food safety and diagnostics has a great socio-economic significance. The detection methodology of biosensors is quite varying and ranges from optical (e.g., fluorescence detection, SPR, interferometry, optical

grating) and piezoelectric to electrochemical (e.g., amperometric, impedimetric, voltammetric) transducers (Becker and Cooper, 2011; Cooper, 2002; Hintsche et al., 1994; Homola, 2006; Sheikh et al., 2008; Turner, 2000). High sensitivity, selectivity, rapid analysis, the ability to operate in turbid solutions and the possibility of miniaturisation enabled electrochemical biosensors to become the most widely used biosensors (Shah and Wilkins, 2003) (Newman and Turner, 2005). The design of an electrochemical biosensor involves careful consideration of many parameters such as electrode design, sensor surface chemistry, recognition element immobilisation on electrode surface, assay conditions and enzyme/mediator selection (Borgmann et al., 2011).

Despite the numerous advantages of electrochemical sensors and many years of scientists' efforts, important hurdles in their development still remain. A key issue that needs to be addressed is the development of electrode probes that can be fabricated into useful arrays for multiplex detection (Drummond et al., 2003). Electrochemical measurements usually involve a three electrode system that consists of a working, counter and reference electrode.

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Traditional electrochemical measurements involve the use of Ag/AgCl as the reference electrode. This functions as a redox electrode for the reaction between Ag metal and its salt, AgCl. This reference electrode is usually formed by a glass tube containing a silver wire that is coated with a thin layer of silver chloride (Czaban and Rechnitz 1976). Although this reference electrode provides fast electrode kinetics, it is not practical when portable, smaller and single use electrochemical biosensors are required. Thus, a new type of electrochemical chip that comprises planar electrodes in the form of either screen printed electrodes (SPEs) or thin film electrodes has gained widespread use. SPEs are printed using inks (carbon or metals together with a polymer matrix) on plastic or ceramic substrates (Hart and Wring, 1994) and thin film electrodes are deposited on glass or silicon wafers by means of evaporation or sputtering methods. Thin film technologies enable the manufacturing of electrodes with high precision and resolution. If micron sized electrodes need to be produced a photolithography process is required (Wu et al., 1993); otherwise a fine metal mask is used to create the electrode patterns on substrates (Zhou et al., 2003). By a sequential process, different metal inks can be printed on SPE; although carbon and gold inks are the most widely used working or counter electrode materials, as pseudo-reference electrodes, Ag/AgCl ink is widely used. However, in the case of thin film electrodes, it is not possible to evaporate or sputter Ag/AgCl to form a reference electrode. Therefore, for thin film electrodes, as an alternative, application of quasi-reference electrodes in the form of Au or Pt has gained some use. While some studies exist that investigate the usability of Ag/AgCl pseudo electrodes, the studies that examine the effects of quasi-reference electrode on electrochemical measurements are rather limited (Kasem and Jones, 2008).

A number of studies have been performed to assess the electrochemical properties of microelectrodes with different geometries and sizes (Guo and Lindner, 2009; Kurita et al., 2000). From these studies it was observed that, although microelectrodes do have several advantages over macro-electrodes (Stett et al., 2003), such as higher current density, smaller sensor footprint, and higher diffusion coefficient, the most obvious disadvantage includes their higher impedance due to interfacial capacitance, which results in very low currents (within or below nano-ampere range) (Ordeig et al., 2008) and their need for expensive and time consuming fabrication involving photolithography (Fiaccabrino and Koudelka-Hep, 1998). Especially if single use sensors are considered for end user applications such as diagnostics, food or environmental testing, cheaper and less time consuming production procedures need to be considered. For this reason, in the current study a novel electrode array has been designed and characterised in order to create a sensor chip which is not only easy, rapid and cheaper to produce but also has a smaller imprint and good electrochemical sensing properties. The current study includes the assessment of the effects of an Au quasi-reference electrode and the use of shared reference/counter electrodes for the array. In addition, this study investigates the electrochemical performance of new electrode arrays by means of an enzyme (horse radish peroxidase, HRP) and bisphenol A detection assays.

2. Materials and instrumentation

Phosphate buffered saline (PBS, 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4) tablets, mercaptoethanol, mercaptoundecanoic acid (MUDA), ethanolamine, spectrophotometric grade ethanol, horse radish peroxidase (HRP), TMB ready to use reagent (containing H₂O₂), bisphenol A and potassium ferricyanide were purchased from Sigma-Aldrich (Poole, UK). Potassium chloride (KCl) was purchased from Fisher Scientific

(Loughborough, UK). Oxygen free argon was purchased from Habas (İstanbul, Turkey). Ultrapure water (18 MΩ/cm) was obtained from a Milli-Q water system (Millipore Corp., Tokyo, Japan).

3. Methods

3.1. Transducer fabrication

Three electrode arrays were designed. **Design 1** consists of eight Au electrodes of different sizes (diameter sizes: 1.5, 2, 3, and 4 mm) and each has its own Au counter and quasi-reference electrode. **Design 2** consists of eight Au electrodes of different sizes (diameter sizes: 1.5, 2, 3, and 4 mm) and all share the same Au counter and quasi-reference electrodes. **Design 3** consists of eight Au electrodes of 1.5 mm diameter and all share the same Au counter and quasi-reference electrodes. An electron beam evaporator device was used to deposit Ti and Au metals on the glass slides. The designs of the electrodes were formed on the glass slides by means of Fine Metal Masks made of a laser cut patterned stainless steel. Before the application of Au (200 nm), a 20 nm Ti layer is applied on to the fine glass slides as an intermediary adhesive layer to increase the adhesion between Au and glass slide. A flow cell was designed and fabricated using PMMA to be used for the electrode arrays.

3.2. Electrochemical analysis

Cyclic voltammetry measurements were performed with a Dropsens MicroStat 8000 Electrochemical Analyzer with the general purpose electrochemical software Dropview 1.4 (Dropsens, Asturias, Spain). The electrochemical analyzer and the purpose built shielded cables enable simultaneous electrochemical measurements of eight electrodes. Cyclic voltammetry (CV) tests were performed using 1 mM potassium ferricyanide solution in 1 M KCl.

3.3. Assay

Bisphenol A detection assay was performed by injecting bisphenol A (in PBS) at varying concentrations on to the plasma cleaned bare Au electrode array. After bisphenol A injection, the flow stopped and 0.5 V potential has been applied to the electrode arrays for 60 s and the current vs. time plot has been obtained. The current value at the 60th second of the test has been recorded as sensor response. For the horse radish peroxidase (HRP) assays, initially plasma cleaned bare Au electrode arrays were immersed in ethanolic solution of 2 mM mercaptoundecanoic acid (80%) and 2 mM mercaptoethanol (20%) mixture for overnight. Later the electrode arrays were rinsed with ethanol and water. After drying with nitrogen stream, the arrays were stored at +4 °C till use. Enzyme assays were performed by mixing HRP and TMB reagent, then injecting on to the flow cell containing electrode arrays (Fig. 1). The chronoamperometric responses obtained at –0.1 V potential at 60 s of the measurements were used as assay response.

Three data points were used to obtain the mean and standard deviation of the results. The limit of detection (LOD) was calculated as the signal obtained from the assays that is equivalent to three times the standard deviation of the signals obtained from the blank standards.

4. Results and discussion

4.1. Cyclic voltammetry

To investigate the electrochemical behaviour of the designed electrode arrays, a cyclic voltammetry technique has been utilised

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