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Fully disposable microfluidic electrochemical device for detection of estrogen receptor alpha breast cancer biomarker

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ABSTRACT

A novel fully disposable microfluidic electrochemical array device (μFED) was developed and successfully applied for detection of the biomarker estrogen receptor alpha (ERα). The μFED was constructed using low-cost materials and an inexpensive home cutter printer enabled the manufacture of dozens of μFEDs in less than 2 h, at a cost of less than US$ 0.20 in material per device. The μFED incorporates counter and reference electrodes and eight carbon-based working electrodes, which were modified with DNA sequences known as estrogen response elements (DNA-ERE), where ERα binds specifically. Paramagnetic particles heavily decorated with anti-ERα antibody and horseradish peroxidase (MP-Ab-HRP) were used to efficiently capture ERα from the sample solution. The ERα-MP-Ab-HRP bioconjugate formed was injected into the μFED and incubated with the DNA-ERE-modified electrodes, followed by amperometric detection with application of −0.2 V *vs.* Ag|AgCl while a mixture of H_2O_2 and hydroquinone was injected into the microfluidic device. An ultralow limit of detection of 10.0 fg mL^{-1} was obtained with the proposed method. The performance of the assay, in terms of sensitivity and reproducibility, was studied using undiluted calf serum, and excellent recoveries in the range of 94.7–108% were achieved for the detection of ERα in MCF-7 cell lysate. The μFED system can be easily constructed and applied for multiplex biomarker detection, making the device an excellent cost-effective alternative for cancer diagnosis, especially in developing countries.

1. Introduction

A biomarker can be defined as a parameter that can be measured and evaluated as an indicator of a normal physiological process, a pathological process, or a pharmaceutical response to a therapeutic intervention (Cummings et al., 2008). Considerable effort has been directed towards identifying biomarkers able to detect cancer at an early stage, or to guide selection of the best therapy for a specific patient (Gutman and Kessler, 2006). Breast cancer, the most commonly diagnosed cancer among women, is a complex and heterogeneous disorder and may consist of several subtypes with different molecular profiles and biological behaviors (Weiderpass et al., 2011).

The estrogen receptor alpha (ERα) biomarker is a nuclear hormone receptor and transcription factor. It regulates the expression of genes and affects cell proliferation and differentiation in the target tissue. The presence of high levels of ERα in breast epithelium may indicate an increased risk of breast cancer, suggesting a role of ERα in the initiation as well as the progression of cancer (Ali and Coombes, 2000). This biomarker is overexpressed in 50–80% of breast cancer cases. It is usually detected by immunohistochemical techniques that involve locating specific antigens in cells or tissues, based on antigen-antibody recognition. ER-positive tumors tend to have a better prognosis than those that are ER-negative, at least for the first 5–6 years after initial diagnosis, and they are also more likely to respond to hormonal therapy employing a targeted hormonal agent, such as tamoxifen or an aromatase inhibitor (Ludwig and Weinstein, 2005).

Although immunohistochemistry is widely used for detection of ERα, there are significant disadvantages associated with the technique, including aspects related to storage, analysis time, and type of sample fixation, as well as the intensity of antigen retrieval. The use of different staining procedures and varied methods of interpreting results can lead to errors such as false negatives (Allred, 2010). In order to overcome the problems associated with immunohistochemistry analysis, several new technologies have been developed and validated for the determi-

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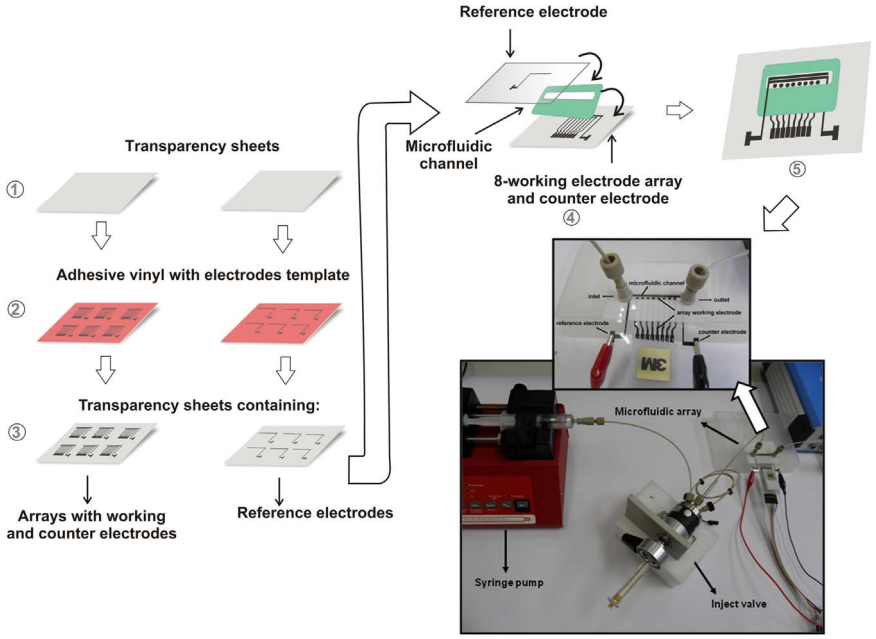

Fig. 1. Construction of the μFED. Screen-printed electrodes: 1) transparency sheets, 2) transfer of vinyl masks to the transparency sheets and carbon ink screen-printing, followed by Ag|AgCl ink deposition on the RE; 3) vinyl mask removal. Assembly of the μFED: 4) sandwiching of the microfluidic channel between the polyester sheets, compressing the RE and 8-WE/CE sheets; 5) μFED ready for use, and image of the microfluidic setup.

nation of ERα, including PCR (Al-Bader et al., 2010), gene-expression microarrays (Oh et al., 2006), and silicon nanowire biosensors (Zhang et al., 2011, 2010), among others (Ahirwar et al., 2016; Lacroix et al., 2001; Padmanabhan et al., 2010). A highly selective and low-cost option for rapid and accurate detection of tumor biomarkers involves the use of biosensors and point-of-care technologies (Amouzadeh Tabrizi et al., 2017; Cabral et al., 2016; Soper et al., 2006; Tang et al., 2010, 2011). In this context, only one paper published in the literature has reported the electrochemical detection of ERα using a biosensor (Eletxigerra et al., 2016). This employed a magnetoimmunosensor with commercial screen-printed carbon electrodes and a sandwich configuration based on antibodies for the determination of ERα, achieving a detection limit of 19 pg mL^{-1}.

The development of point-of-care methods based on microfluidic systems brings benefits including the ability to rapidly obtain results using portable devices that require only small volumes of reagents and samples, produce minimal waste, and can be automated and miniaturized (Henry et al., 2009; Lin et al., 2017, 2016; Pakchin et al., 2017; Whitesides, 2006). However, despite these advantages, the construction of microfluidic systems is not trivial. It can involve photolithography techniques that require clean rooms and qualified personnel (Haraldsson et al., 2006; Kim et al., 2008); when polymethylmethacrylate (PMMA) is used for construction of the systems, screws or magnets are needed to fix the parts (Krause et al., 2013; Otieno et al., 2014; Sardesai et al., 2013; Wasalathanthri et al., 2013; Zheng et al., 2009); the manufacture of devices using low temperature co-fired ceramics (LTCC) is time-consuming and requires high temperatures (Pessoa-Neto et al., 2014; Suarez et al., 2010).

More recently, 3D printers have been used to construct microfluidic devices, but this usually requires the use of external electrodes, since they cannot be printed directly (Gabardo and Soleymani, 2016; Ho et al., 2015). Some systems employ curable materials, but this results in highly complex construction in order to ensure adhesion between the substrates (Hamad et al., 2016). The fabrication of other systems, despite being based on inexpensive materials, involves the use of rigid materials and the molding of microchannels, which makes them fragile and/or only permits semi-quantitative detection of analytes (Martin et al., 2016). The reuse of these devices usually requires repetitive steps of disassembly, cleaning, and reassembly between measurements, which increase the analysis time and the risk of false negative or positive results due to contamination of the system.

This work describes the construction of an innovative fully disposable microfluidic device (μFED), consisting of an array of electrodes, for the fast and selective detection of ERα. The microarray of eight working electrodes was constructed with low-cost materials, employing an inexpensive home cutter printer. This device offers flexibility, disposability, portability, simplicity of construction, ease of use, and suitability for large-scale production. The μFED was applied for the detection of ERα in undiluted calf serum and in MCF-7 cell lysates, providing ultrahigh sensitivity.

2. Materials and methods

2.1. Chemicals and materials

Horseradish peroxidase (HRP), reduced L-glutathione (GSH, 99%), gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9%), sodium borohydride (99%), poly(diallyldimethylammonium chloride) (PDDA), bovine serum albumin (BSA), bovine calf serum, 1-(3-dimethylaminopropyl)–3-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), hydrogen peroxide (30%), Tween-20, ferrocenecarboxylic acid (97%), and hydroquinone (HQ, ≥99%) were purchased from Sigma-Aldrich. Carboxyl functionalized superparamagnetic particles (~1.5 μm) were acquired from Polyscience Inc., and recombinant human estrogen receptor alpha and polyclonal anti-ERα antibody (Ab) were purchased from Imuny Inc., Brazil. ERα stock solution was prepared at a concentration of 0.2 mg mL^{-1} in undiluted calf serum. Aminated double-stranded DNA (DNA-ERE) composed of a sequence of estrogen response elements was purchased from Sigma-Aldrich. The forward sequence of the DNA-ERE was 5′-H_2N-GTCCAAAGTCAGGTCACAGTGACCTGATCAAAGT-3′ (Klinge, 2001; Tan et al., 2010)

2.2. Fabrication of the μFED

The μFED was constructed in two steps. Firstly, the array of 8 carbon-based working electrodes (8-WE), the pseudo-reference electrode (RE), and the counter electrode (CE) were constructed using a