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Microfluidic immunosensor based on mesoporous silica platform and CMK-3/poly-acrylamide-*co*-methacrylate of dihydrolipoic acid modified gold electrode for cancer biomarker detection

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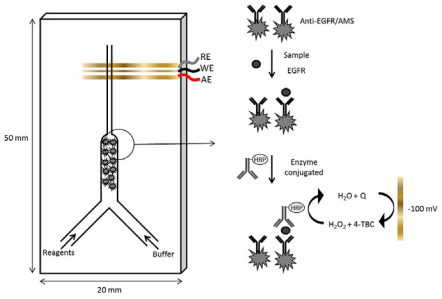
HIGHLIGHTS

- A microfluidic immunosensor has been developed for epidermal growth factor receptor (EGFR) in human serum samples.
- In this immunoassay, the anti-EGFR antibodies are immobilized on a surface area amino mesoporous silica as platform.
- The gold electrodes are coated with CMK-3/poly-acrylamide-co-methacrylate of dihydrolipoic acid nanocomposite.
- The sensor's linear range is from 0.01 to 50 ng mL^{-1} with a single day RSD of 4.98%.

GRAPHICAL ABSTRACT



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ABSTRACT

We report a hybrid glass-poly (dimethylsiloxane) microfluidic immunosensor for epidermal growth factor receptor (EGFR) determination, based on the covalent immobilization of anti-EGFR antibody (anti-EGFR) on amino-functionalized mesoporous silica (AMS) retained in the central channel of a microfluidic device. The synthetized AMS was characterized by N_2 adsorption-desorption isotherm, scanning electron microscopy (SEM), energy dispersive spectrometry (EDS) and infrared spectroscopy. The cancer biomarker was quantified in human serum samples by a direct sandwich immunoassay measuring through a horseradish peroxidase-conjugated anti-EGFR. The enzymatic product was detected at −100 mV by amperometry on a sputtering gold electrode, modified with an ordered mesoporous carbon (CMK-3) in a matrix of poly-acrylamide-*co*-methacrylate of dihydrolipoic acid (poly(AC-*co*-MDHLA)) through *in situ* copolymerization. CMK-3/poly(AC-*co*-MDHLA)/gold was characterized by cyclic voltammetry, EDS and SEM. The measured current was directly proportional to the level of EGFR in human serum samples. The linear range was from 0.01 ng mL^{-1} to 50 ng mL^{-1}. The detection limit was

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Microfluidic immunosensor
Nanostructured electrode

3.03 pg mL^{-1}, and the within- and between-assay coefficients of variation were below 5.20%. The microfluidic immunosensor is a very promising device for the diagnosis of several kinds of epithelial origin carcinomas.

1. Introduction

Epidermal growth factor receptor (EGFR, ErbB1; HER1) is a cell *trans*-membrane protein located on the cell surface, that is activated by binding to their specific ligands, such as epidermal growth factor and transforming growth factor, which activates the tyrosine kinase in its intracellular domain and induces intracellular signaling pathways that control relevant cellular processes, including adhesion, migration, apoptosis, cell differentiation and proliferation [1,2]. Overexpression of EGFR protein has been reported in several kinds of epithelial origin carcinomas, such as breast, lung, head/neck, gastric, colorectal, renal, prostate, esophageal, ovarian, and pancreatic cancers [3,4]. Furthermore, the increased expression of EGFR is linked to an increased rate of proliferation, increased invasiveness and a high mortality prognostic. Hence, there is increasing interest in the quantification of EGFR levels, both for diagnosis and prognosis as an indicator associated with more aggressive and resistant to treatment tumors [5,6].

Conventional techniques for EGFR determination include enzyme-linked immunosorbent assay (ELISA) of intact cells [7,8], western blotting of cell lysate [9,10], and immunohistochemistry on tumor tissue samples [11,12]. However, these methods are technically demanding, time consuming and require highly qualified personnel. Thus, the development of a sensitive, selective, simple, rapid, and inexpensive method for detecting cancer biomarkers in real samples is critical.

To improve the detection of EGFR, the sample delivery and sample preparation capabilities of microfluidic devices have been utilized to greatly increase the speed of EGFR detection using immunostaining of whole cells. These devices capture cells above a track-etched membrane and stain the cells before characterization using fluorescence microscopy [17,18]. Recently, the electrochemical immunosensors have become more important for the detection of cancer biomarkers [13,14], due to the electrochemical detection provides the required limits of detection, selectivity, rapid analysis times, and faster data analysis than immunostaining methods. These electrochemical immunosensors can be readily incorporated into microfluidic devices, which offer several useful properties including low sample and reagent consumption, high sensitivity, fast response, portability, ease of use and short time for analysis [15,16].

Of the numerous methods available to fabricate microfluidic devices, the hybrid glass-poly (dimethylsiloxane) (PDMS) microfluidic devices are particularly well-suited for integration of electrochemical devices. The glass substrate provides a support that is thermally stable and amenable to deposition of the electrodes by sputtering. PDMS based microfabrication methods are well-established and provide an inexpensive method for rapid reproduction of the layer with patterned substrates. Therefore, these hybrid devices utilize the best attributes of both substrates and also have the advantages of: low cost, durability, chemical inertness, optical transparency, and automation capacity [19,20]. In addition, electrochemical detection allows great versatility, which can be easily incorporated into a portable device. The gold electrodes do not require vapor deposition and can be easily sputtered on the glass [21,22].

To improve the limit of detection several publications reported the use of simple/multiwalled carbon nanotubes, graphene, carbon nanofibers among others [23,24]. These materials have unique electric, electrocatalytic and mechanical properties, which make them efficient materials for developing electrochemical immunosensors. However, we increased the surface area of the gold electrode through *in situ* copolymerization and deposition of an ordered mesoporous carbon (CMK-3) in a matrix of poly-acrylamide-*co*-methacrylate of dihydrolipoic acid (Poly(AC-*co*-MDHLA)). CMK-3 has been used in different applications due to the periodically mesoporous structure, high specific surface area, large pore volume, and tunable pore size distribution [25]. These materials have been widely used, because of their fast electron transfer, high surface area, and excellent electrocatalytic activity, avoiding surface fouling. The excellent electrocatalytic properties of modified electrodes with ordered mesoporous carbons have been reported [26]. Furthermore, the poly(AC-*co*-MDHLA) has thiol functional groups that bind strongly to gold, thereby obtaining a highly stable electrode under extreme conditions of salt and pH [27].

In the last years, different materials have been incorporated into electrochemical immunosensors as immobilization supports for the specific immunoreactants. One the most commonly used solid supports are the different kinds of porous silica materials [28,29]. These porous silica materials have many benefits, such as the increase of the surface to volume ratio that increases interactions between the immunoreagents and EGFR and lowers the limits of detection [30,31]. We synthetized, functionalized, characterized and used an amino mesoporous silica (AMS) as immobilization platform for the anti-EGFR antibody (anti-EGFR). AMS has an increased surface and uniform porous, compared with the commercial 3-aminopropyl modified controlled pore glass (AP-CPG) normally used as immobilization support.

In the present work, we developed a hybrid glass-PDMS microfluidic electrochemical immunosensor for EGFR determination, based on the covalently immobilization of anti-EGFR on AMS retained in the central channel (CC) of the microfluidic device. AMS was characterized by N_2 adsorption-desorption isotherm, scanning electron microscopy (SEM), energy dispersive spectrometry (EDS), and infrared spectroscopy (FTIR). The cancer biomarker was quantified by a direct sandwich immunoassay measuring through a horseradish peroxidase (HRP)-conjugated anti-EGFR. The enzymatic product (benzoquinone) was detected by reduction at −100 mV by amperometry on a sputtering gold electrode modified with CMK-3 in a matrix of poly(AC-*co*-MDHLA) through *in situ* copolymerization. CMK-3/poly(AC-*co*-MDHLA)/gold was characterized by cyclic voltammetry (CV), EDS and SEM. The measured current was directly proportional to the level of EGFR in human serum samples. To the best of our knowledge, no study involving a microfluidic electrochemical immunosensor with an own design, using AMS as immobilization platform for EGFR determination in biological samples has been reported being this the novelty of our work. The microfluidic immunosensor is a very promising device for the diagnosis of several kinds of epithelial origin carcinomas.