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Biomicrosystem design and fabrication for the human papilloma virus 16 detection

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ABSTRACT

In this paper, a biomicrosystem consisting of 98 biosensors based on the monoclonal antibody (mAb) 5051 immobilization for the detection of the human papilloma virus (HPV) 16 was designed and fabricated. The mAb 5051 immobilization was performed on a self-assembled monolayer of 4-aminothiophenol, on a polymethylmethacrylate substrate with a gold nanolayer. The biomicrosystem carries out 98 in situ different simultaneous tests without specialized equipment or personnel. The biomicrosystem characterization was performed by electrochemical impedance spectroscopy, cyclic voltammetry, impedance measurements, AFM and SEM. The biomicrosystem performance was verified by using positive and negative HPV 16 real samples from women, after 1 h incubation process using 40 biosensors from 4 different biomicrosystems. The HPV 16 samples were verified by a standard PCR test. An analytic hierarchy process was performed based on costs, effectiveness, time and operative characteristics between the biomicrosystem and traditional techniques for the HPV detection, such as PCR-RT and Hybrid Capture 2; concluding that the biomicrosystem is a dominant alternative with the 38.8% in the global score compared to the 34.5% of PCR-RT and the 26.7% of HC2.

1. Introduction

Biosensors are devices used for detection and biological recognition based on the immobilization of biomolecules on surfaces of transduction, creating a functional sensor. The immobilization of biomolecules is fundamental for the functionality and integrity of the biosensor, because the biomolecule orientation facilitates the union with the objective analyte. A steric impediment or the denaturalization of the bio-receptor is a problem for the reproducibility and the sensibility of the biosensor [1]. Immune-sensors are biosensors of affinity based on antibodies as elements of bio-recognition, due to the specificity of antibodies to link a certain protein, another antibody, or even an entire virus with a high affinity [1–3].

Nowadays, the implementation of SAMs (self-assembled monolayers) technology, offers one of the simplest ways to provide a suitable cap of orientated immobilization of antibodies [2,4,5]. SAMs are formed in a spontaneous way from the adsorption of a substance with specific affinity to a substrate, generating organized molecular structures [6,7]. One of the more studied self-organized systems in the last years, consists of alkanethiols monolayers (CH3CHn-SH) on gold thin layers [8,9]. Alkanethiols have a high affinity to golden surface, which properties of conductivity, reflectivity and affinity to react in a spontaneous way, allow to control the quantity and the orientation of the immobilized antibodies; where the site of recognition is available for a major accessibility of the antigens, allowing the kinetic interaction and recognition of the objective analyte to be less variable [6,10].

The use of microsystems has had an increased interest in the detection of analytes; and new technologies of micromanufacturing have been introduced for mass production [11]. The main advantages of microsystems are: short time of response, field detection capacity, portability and low cost analysis, due to the minimal quantity of reagents needed given its size [12].

Moreover, human papilloma virus (HPV) is a DNA virus of double chain and the viral pathogen most commonly sexually transmitted. It infects epithelial cells and is associated with cervical lesions, candylomas and cervical cancer [13]. There are more than 100 types of HPV. Some of these are low risk types that are associate with the production of genital warts: HPV 6 and HPV 11, and the high risk HPV's, which are associated with the development of cervical

Abbreviations: 4-ATP, 4-aminothiophenol; AC, alternate current; AFM, atomic force microscope; CV, cyclic voltammetry; DC, direct current; EIS, electrochemical impedance spectroscopy; HC2, Hybrid Capture 2; HPV, human papilloma virus; mAb5051, monoclonal antibody 5051; PAJ, analytic hierarchy process; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PCR-RT, chain reaction polymerase in real-time; PMMA, polymethylmethacrylate; SAMs, self-assembled monolayers.

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cancer: HPV 16 and HPV 18; being the HPV 16 is the most prevalent [14,15]. Nowadays, two molecular assays are implemented to diagnose and classify: Hybrid Capture and polymerase chain reaction (PCR) [16]. Although these methods are effective, they are limited by the time needed to obtain a diagnosis, because of the preparation and purification of the sample before performing the analysis and the need of staff, equipment and specialized laboratories. As an alternative to these technologies, the design and manufacture of microsystems for specific analysis has gained attention. Du et al. [17] developed a microfluidic platform using the antibody α6-integrin, where cervical cancer cells were recognized. The α6-integrin was used as a capture antibody bound, since the HPV-16 cervical cancer cells and the α6-integrin cell surface are correlated. In this study, more than 30% of the cells that were captured were cancer cells and just less than the 5% were normal cells; however, the platform has to be used in lab facilities [17]. Mata et al. [18] fabricated a low cost microsystem to detect *Salmonella enterica* CECT 724 pathogens from different samples. They immobilized paramagnetic immunoparticles inside the microsystem for the microorganisms capture, and verified it through electrochemical impedance and amperometric methods in less than 30 min after the sample was introduced. These techniques implied the use of lab-grade equipment [18]. Moreover, Towne et al. [19] performed a study to detect HPV-16 using surface plasmon resonance technology involving the direct immobilization of anti-human papilloma virus (HPV) type 16 mAbs to the surface of dextran-coated sensor chips. This technique has an advantage over the indirect capture such as the via rabbit antimouse Fc process, since it does not need additional stages to for the unoccupied immobilized anti-Fc blocking, to prevent non-specific antibody binding. This method is useful for characterizing consistency for process changes that may alter product antigenicity, but also requires specialized lab equipment [19]. In this work, we present the design and manufacture of a portable biomicrosystem, which consists of 98 integrated biosensors. The biosensors are based on a self-assembled monolayer of 4-Aminothiophenol (4-ATP) for the detection of the HPV 16 by the immobilization of the antibody monoclonal 5051. The biomicrosystem, is simple to manufacture, its use does not need specialized personnel, and allows carrying out 98 tests in situ simultaneously.

2. Materials and methods

2.1. Reagents and equipment

Glass slides of 25 mm × 75 mm and 1 mm thickness were bought from Soluquin S.A.S. (Colombia), polymethylmethacrylate (PMMA) slides of thickness 2 mm of the same size and the methylene chloride were bought from Diacrílicos J.D. (Colombia). Gold at 99.9% was acquired in Kurt J. Lesker (USA), 4-ATP at 97%, ethanol, NaCl, KCl, Na_2HPO_4, K_2HPO_4, NaOH and the HCl were obtained from Sigma-Aldrich (USA). The mAb 5051 was acquired from ThermoScientific (USA) and the electrical connectors from Contactos y Empaques LTDA (Colombia). A Phosphate buffered saline (PBS) was prepared using 130 mM NaCl, 20 mM KCl, 8 mM Na_2HPO_4 and 11 mM K_2HPO_4 for the immobilization process. pH was adjusted to 7.2 using NaOH or HCl.

The different designs of the PMMA slides, were fabricated with a laser cutting machine CF 1306 (Katianlaser, USA). For the gold depositions, a thermal evaporator Edwards E306 (Moorfield, UK) and a metallizer Denton vacuum Desk IV (Moorestown, USA) were used.

Measurements for the biomicrosystem were carried out with a potentiostat PGSTAT128N (Metrohm, USA), an impedance analyzer Agilent 4294A (Agilent Technologies, USA), a digital multimeter PeakTech Multifunction Tester 3725 (PeakTech, Germany), an oscilloscope OSCI8489 (Tektronix, USA), a signal generator HM8130 (Hameg, Spain), a digital multimeter Fluke 79 III (Fluke, USA) and an impedance meter SR 715 LCR (Stanford Research Systems, USA). The immobilized surfaces observations were performed with a scanning electron microscope (SEM) Phenom G2 pro desktop SEM (Phenom-World, The Netherlands) and an atomic force microscope (AFM) Mfp 3D BIO ac 240 ts (Olympus Asylum Research, USA).

2.2. Gold deposition

For the substrate fabrication (glass or PMMA) with gold, a thermal evaporator with 2.8 A was used over a tungsten slide, for the 90 mg gold evaporation. A vacuum pressure of 4×10^{-5} mbar and an evaporation rate of 0.3 nm/min were established. In addition, a metallizer was used with a vacuum pressure below the 100 mTorr, 41 mA and 660 s of exposition time. Gold nanolayers between 35 and 80 nm were obtained.

2.3. Biomicrosystem development

On the substrate covered with gold (Fig. 1(B)), a slide with wells organized in 7 rows and 14 columns of diameter 2 mm, horizontal interconnected was set (Fig. 1(C)). This first slide had as objective a better adhesion to the substrate, in order to avoid the leakage of reagents, thus, it was fabricated with a higher surface area. On this first slide, two slides with wells in 7 rows and 14 columns of diameter 2 mm, were pasted with methylene chloride. In those wells, reagents were deposited for the biosensor development. Wells were embedded by square holes of 0.6 mm side, where electrical connectors were placed for electrical measures (Fig. 1(E)). Each biomicrosystem fabricated contained a total of 98 biosensors. A total of 5 biomicrosystems were fabricated and tested.

2.4. Determination of the 4-ATP and mAb 5051 solution concentration

An experimental design was performed with 2 factors and 3 levels of factors: 4-ATP (10, 15 and 20 mM) concentration and mAb 5051 (50, 75 and 100 μg/ml) concentration, in order to determine the optimal concentrations to develop the self-assembled layer, For the mAb 5051 immobilization, 4 μL of each of the 4-ATP solutions were added into the wells of 18.85 μL. The solutions reacted during 4 h and then were washed with ethanol and deionized water, in order to remove remaining thiols. Then, 4 μL of each of the mAb 5051 solutions, were incubated for 1 h at 37 °C and were washed with PBS and deionized water.

Finally, impedance measurements, Electrochemical Impedance Spectroscopy (EIS) and cyclic voltammetry (CV) were performed to determine the optimal concentrations for the immobilization, based on greater changes in the impedance measures.

2.5. Electrochemical impedance spectroscopy and cyclic voltammetry

EIS and CV measures were performed for the mAb 5051 immobilization testing. For these measurements, 20 ml of $Fe(CN)_6^{3-/4-}$ 1 mM that contained 0.1 M PBS (pH 7.4) was prepared. A potential between −0.2 and 0.6 V was applied vs $Ag|AgCl|KCl_{3M}$ with 100 mV/s of scanning speed. The frequency range for EIS was set from 0.1 Hz to 100 kHz and a sinusoidal modulation potential of ±5 mV was overlapped in the 0.2 V DC potential vs $Ag|AgCl|KCl_{3M}$. Before each experiment, the solution was deoxygenated by bubbling high purity nitrogen for 10 min. Impedance data were adjusted to an equivalent circuit, using ZView (Scribner Associates,