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Peptide modified paper based impedimetric immunoassay with nanocomposite electrodes as a point-of-care testing of Alpha-fetoprotein in human serum

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ABSTRACT

Treatment for cancer depends on the type of cancer, and the stage or its development, and thus the need for point-of-care technology that can allow rapid and precise detection of biomarkers is increasing. Here, we present a simple on chip electrical detection of Alpha-fetoprotein (AFP). We rely on using a novel peptide modified plastic-paper microfluidic chips to perform efficient and specific impedimetric detection of AFP in human serum. The chips are prepared from a lower sheet of plastic and upper layer of cellulose chromatography paper modified with silver-20 wt% graphene printed electrodes. Diphenylalanine (FF) was proposed to involve in detection zone of the fabricated microchips in order to improve the sensing performance and the stability of immobilized antibodies according to amine-aldehyde reaction. The target protein is captured on the surface of microchips using specific monoclonal antibodies and the electrical response of the chip is monitored in the presence and absence of different concentrations of AFP. The influence of several parameters including the material types for screen printing of electrodes, FF concentrations, solvent and pH of FF solution on electrical response and cellulose fibers morphology was explored. The impedance measurements of AFP on the fabricated microchip in the optimized parameters exhibited a detection limit of 1 and 10 ng ml^{-1} in PBS and plasma, respectively. This platform developed here can be adopted to develop systems for rapid detection of biomarkers using portable electric devices.

1. Introduction

In the recent years, studies represent the prevalence of infants born with a particular congenital disability like Down syndrome has increased dramatically with an annual growth rate of 0.9%. Moreover, Hepatocellular Carcinoma (HCC) is one of the worldwide common malignancies generally followed by liver cirrhosis or chronic infection with hepatitis B or C virus (Karamouzis et al., 2003). HCC accounts for 70–80% of all liver cancers, and although most liver cancer (83%) was diagnosed in less well-developed nations in South-Asian countries and Africa (Wong et al., 2017; Kew, 2012), the percentage of people in the United States who get liver cancer has been increasing for several decades. Every year, about 70% of men and 90% of women of Americans who get liver cancer, die from the disease (Group, 2010). Alpha Feto Protein (AFP) is a protein made in the liver of a fetus, and the amount of it in a pregnant woman blood can help to see whether the fetus may have such problems as spina bifida, anencephaly, and omphalocele (Ball et al., 1992). Also, AFP level in the serum of pregnant woman's blood can be checked to find chromosomal problems, such as Down syndrome (trisomy 21) or Edwards syndrome (trisomy 18) (Muller et al., 2003). In adults, AFP is considered as a tumor marker to help detect and diagnose cancers of the liver (Otsuru et al., 1988). The AFP level is often ordered to monitor in people with chronic liver diseases such as cirrhosis, chronic hepatitis B or hepatitis C.

Therefore, for early detection and clinical follow-up of patients with hepatocellular carcinoma, and in the case of a pregnant woman, alpha-fetoprotein (AFP) is the first serologic biomarker (Bird et al., 2016), and it has been considered as one of the most important biomarkers in diagnosing and targeting of hepatocellular carcinoma and birth defects in a developing baby (Otsuru et al., 1988; Kronquist et al., 1990). In the past few years, a number of methods have been reported for detection of AFP such as electrochemiluminescence (Zheng et al., 2016; Chen et al., 2011), electrochemical immunoassay (Feng et al., 2015; Wang et al., 2017; Kavosi et al., 2014), enzyme-linked immunosorbent assay

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(ELISA) (Liu et al., 2013; Lee et al., 2009), radioimmunoassay (Chester et al., 1991), fluorescence biosensor (Jiang et al., 2016) electrophoretic immunoassay (Liu et al., 2017), and surface-enhanced Raman scattering immunoassay (Li et al., 2013).

Due to the time-consuming, problems in mass production, cost, disposing and the complexity of laboratory technics and requiring qualified technicians to handle samples and analyze results, the clinical applications have been restricted. To address these limitations, Lab-on-a-Chip Systems were suggested (Whitesides, 2006). Glass and silicon based chips were the first systems; however, the invention of new materials such as plastic derivatives provides the construction of disposable systems with remarkable properties (Harrison et al., 1993; Effenhauser et al., 1993; Duffy et al., 1998).

With the pioneering work of Whitesides' group in 2007, cellulose papers with the high surface area have become an area of growing interest (Martinez et al., 2007). It has been a noteworthy substrate for prototyping new point of care testing (POCT) and has been used extensively as a sensing platform for microfluidic systems in the fields of clinical diagnostics, environmental monitoring, immunoassays, and food and nutrition safety (Li et al., 2012; Ge et al., 2013; Sameenoi et al., 2012; López Marzo et al., 2013). When compared with the conventional microfluidic analytical devices which are fabricated by silicon, glass and super polymer as their substrates, the microfluidic paper-based chips have emerged as a novel systems due to the fact that it is inexpensive, easy to use, transport, store and disposal, ubiquitous and do not require external instruments and complex fabrication processes, abundant, requires small amounts of reagents and allows storing or immobilizing chemicals and compatible with many chemical/biochemical/medical applications (Zhao et al., 2008; Martinez et al., 2008; Bruzewicz et al., 2008).

These characteristics make the paper an ideal platform for the development of microfluidic analytical devices with high sensitivity, low cost, small footprint, portability, simple operation, shorter time of analysis than traditional methods and suitable for application in point-of-care testing. It is an advancing technology particularly relevant to improve the healthcare and disease screening in the developing world, especially for those areas with low-infrastructure and limited trained medical and health professionals. In these systems, a fluid transports spontaneously through the channels using capillary forces without the assistance of external forces. Among these advantages for paper-based microfluidic devices, there are some challenges which need to improve in order to gain more benefits from these systems. In immunoassay paper-based chips, the stability of the immobilized antibodies on paper and the wet-strength of paper are critical. However, an untreated pure cellulose paper will typically lose its strength when saturated in water and offers few functional groups for directly covalent bioconjugation. Also, wet-strength of the microchips is essential during washing steps and handling. For developing desirable functional groups and improving binding of antibodies, for the first time we have introduced a novel strategy using diphenylalanine (FF). This peptide has been extensively investigated because of its unique chemical properties, structural diversity, and biocompatibility, easy to synthesize and modify of this small peptide and design of various nanostructures (Boyle and Woolfson, 2011; Dover et al., 2009; Gong et al., 2015; Sousa et al., 2015; Adler-Abramovich and Gazit, 2014). The electrical conductivity of diphenylalanine based peptide nanostructures has been studied and they have been used in the electrochemistry field owing to their stability and ability to increase the signal/noise ratio (Gong et al., 2015; Castillo et al., 2013; Feyzizarnagh et al., 2016; Cho et al., 2008). On the other hand, when FF is conjugated with other recognition elements used in biosensor including antibodies, they can show a synergistic effect with a high selectivity and sensitivity (Vural et al., 2018). Thus, it is utilized extensively in biosensor applications in the literature. We found that the covalent coupling of antibodies on FF modified microchip possesses high binding-stability and enhanced sensitivity of the microchips consequently. On the other hand, the wet-strength of the paper-based microchip was enhanced by introducing hybrid paper-plastic integrated chips. Therefore, by using FF and producing hybrid paper-plastic microchip, we introduced a novel paper-plastic modification strategy to simultaneously improve the wet-strength for paper microfluidic chips and the stability of immobilized antibodies.

Therefore, to develop a clinical detection and treatment monitoring, an efficient, rapid, inexpensive detection, and specific quantitation of AFP tumor marker in biological samples is an urgent need especially in resource-limited settings. Briefly, in this research, a paper-plastic based immunoassay is proposed for detection of the protein biomarker regarding the high specificity between antibody and antigen. Electrical detection methodologies are expected to underpin the progressive drive towards miniaturized, sensitive and portable biomarker detection protocols. Therefore, by using silver-graphene nanocomposite as a robust biosensing material to develop diagnosis with an electrical sensing modality, we have fabricated high electrical, thermally conductive and flexible electrodes. Based on the specificity of an antigen-antibody interaction, we developed an electrical plastic-paper chip with high sensitivity to detect AFP.

2. Experimental

2.1. Reagent and apparatus

Diphenylalanin in lyophilized form, and 1,1,1,3,3,3-Hexafluoroisopropanol (> 99%) and acetic acid solvents and Phosphate Buffer Saline (PBS) were purchased from BACHEM (Switzerland), Fisher Scientific (Waltham, MA), Merck (Germany) and Sigma-Aldrich (St. Louis, MO), respectively. Alfa fetoprotein (AFP) and anti- AFP antibody was obtained from Lee Bio Solutions (Maryland Heights, MO) and EastCoast Bio (North Berwick, ME)), respectively. Sodium meta-Periodate ($NaIO_4$) and Bovine Serum Albumin (BSA) were purchased from Thermofisher scientific (Waltham, MA) and Life technologies (Carlsbad, CA), respectively. All chemical reagents without further purification were directly used. Whatman chromatography paper (3001-861) was purchased from GE healthcare (Little Chalfont, UK). Silver ink (cl-1001) and conductive graphene dispersion for electrode fabrication were obtained from Engineered Materials System (Delaware, OH), and Graphene supermarket Inc. (Cleverton, NY), respectively.

2.2. Immunoassay fabrication steps

2.2.1. Microchip design and assembling

Schematic representation of chip design and fabrication process is illustrated in Fig. 1. The finger type electrodes microchip was designed to achieve uniform electric field between the electrodes (Fig. 1-a). Fig. 1-b and c show the flexibility and actual dimensions of electrodes and detection zone region. The paper based microchip was prepared of different layers including oxidized cellulose-paper substrate (Whatman filter paper) and flexible plastic sheet (3 M, Italy) bonded using a double sided adhesive (DSA) tape (Fig. 1-d). In order to create a covalent crosslinking between the cellulose fibers and self-assembled FF, it is vital to modify the simple chromatography paper with $NaIO_4$ (0.5 M) and spread carbonyl group on the surface of fibers. In details, Whatman chromatography paper was soaked in $NaIO_4$ solution and kept in dark at room temperature for 30 min. The paper was then washed off three times with DI water by inserting the paper into the water container. After washing step, the paper was dried out using a paper towel and kept in an incubator for 12 h in 37 °C. After surface modification of the paper with aldehyde group, the mask was attached on top of one side of the oxidized cellulose paper and DSA on the other side. The assembled was cut into the electrodes geometry and size using a CO_2 laser cutter (Universal Laser Systems VLS3.50 50). After cutting, the protective layer of DSA side was removed and the plastic sheet was