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On-chip magneto-immunoassay for Alzheimer's biomarker electrochemical detection by using quantum dots as labels

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ABSTRACT

Electrochemical detection of cadmium-selenide/zinc-sulfide (CdSe@ZnS) quantum dots (QDs) as labeling carriers in an assay for apolipoprotein E (ApoE) detection has been evaluated. The immunocomplex was performed by using tosylactivated magnetic beads as preconcentration platform into a flexible hybrid polydimethylsiloxane (PDMS)-polycarbonate (PC) microfluidic chip with integrated screen printed electrodes (SPE). All the immunoassay was performed in chip and in flow mode. The sensitive electrochemical detection was obtained by square wave anodic stripping voltammetry. ApoE was evaluated for its potential as biomarker for Alzheimer's disease detection, achieving a limit of detection (LOD) of $\sim$12.5 ng mL^{-1} with a linear range from 10 to 200 ng mL^{-1} and high accuracy for diluted human plasma.

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1. Introduction

Neurodegenerative processes have a high incidence and prevalence in our society; most of them have no cure and are generally diagnosed in people over 65 years old (Brookmeyer et al., 1998). The evolution of such kind of disease is long and irreversible, whereby the goal of treatment is usually to improve symptoms, relieve pain and increase mobility. One of the most common neurodegenerative illnesses is Alzheimer disease (AD), characterized by pathological changes in the brain, including senile plaques, synapse, neurofibrillary tangles and neuronal loss. AD is usually diagnosed clinically from the patient history, by clinical observation based on neurological features, however there is a wide range of neurological diseases difficult to distinguish, with very long asymptomatic evolution and extensive neurological damages over time that become more difficult to treat, making essential the use of biomarker-based diagnosis for its early stages detection (Reza Mohamadi et al., 2010; Jack et al., 2010).

Biomarkers are indicators of biological status; they can give information about biological processes in normal or pathological states, and even during therapy. One biomarker of interest of AD is Apolipoprotein E (ApoE). ApoE is one of the primary AD polymorphisms, associated not only with risk and age of onset, but also brain integrity in AD (Takeda et al., 2010). ApoE is a 34 kD protein present in the lipid transport and lipoprotein metabolism, whose levels are controlled by the gene ApoE. This biomarker is found in plasma lipoprotein particles, in large amounts at sites of neurological damage, and it is also involved in recycling of apoptotic remnants of amyloidal aggregates. ApoE is mainly produced in the liver and by macrophages but also by cell types including smooth muscle cells and neuronal cells. It exists in three isoforms: ApoE2, ApoE3, and ApoE4. Recently, total ApoE and ApoE4 levels have been reported to be significantly different in AD patients compared to control patients (Kim et al., 2009).

Lab-on-a-chip (LOC) devices have become important tools for sample analysis and treatment with different analyte studies or diagnostics due to benefits such as the reduced sample volume, low cost and portability. Both manufacturers and research laboratories are in search of plastic materials able to take advantage of simple and fast processing methods.

These platforms in combination with nanomaterials offer excellent improvements in properties for many applications (i.e. detectors sensitivity enhancement, biolabeling capability) (Medina-Sánchez et al., 2012a, 2012b). Among this wide variety of nanomaterials,

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quantum dots, also known as semiconductor nanoparticles, which are crystalline clusters of a nanometric size (Murphy, 2002) have special labeling properties. They are most commonly used as labels in imaging and biosensing for their optical properties and their stability; however, QDs have also interesting electrochemical properties. They can be electrochemically detected either by dissolving them liberating the metal ions (Wang et al., 2003) or by direct detection of the QDs (Merkoçi et al., 2007). Furthermore, a wide range of commercial QDs are available, with different surface functional groups, allowing their functionalization with biomolecules such as antibodies, biomarkers, enzymes, DNA or cells. Depending on their structure, this interaction should be considered in the design of the experiments and detection strategy. Thereby, we take the advantages of on-chip magnetic bead manipulation (Pamme, 2006) and link it with the great electrochemical properties of QDs as labels for a sensitive and efficient detection of ApoE. We used magnetic beads as capture/preconcentration platform for improving the immunological reactions (Ambrosi et al., 2011; Zhang et al., 2012; Fang et al., 2011) thanks to their surface area, faster assay kinetics and easy washing steps due to their simple magnetic manipulation by using external magnets.

Magnetic beads into microfluidic platforms for virus (Lien et al., 2011), biomolecules (Lin et al., 2013; Reymond and Vollet, 2013; Teste et al., 2013), cells (Kang et al., 2012) and environmental pollutants immobilization (Zacco et al., 2006) have been widely reported in the literature. These analytes have been labeled mainly with enzymatic and fluorescent (fluorophores) labels for their posterior detection.

On the other hand, the combination of microfluidics and electrochemistry enables quasi automated measurements (Lin et al., 2010; Chikkaveeraiah et al., 2012; Godino et al., 2010). The advantages of these platforms in comparison with standard procedures are: reduced time of incubation steps, low volume consumption, possibility to miniaturize the sample treatment as well as the readout system. This platform could be easily reusable by flushing the channel to remove the particles and introducing fresh solution of beads.

To the best of our knowledge there are not any publications about Alzheimer biomarker detection in microfluidic platforms by using quantum dots as electrochemical labels. In general AD diagnosis has been done by different strategies as it is described in the literature (Akeda et al., 2010). In addition, the development of a microfluidic-based Alzheimer's Aβ aggregates clearance system, suitable for efficient screening of chemical candidates to enhance the clearance of Aβ amyloid deposits prior to *in vivo* analysis was described (Lee and Beum, 2010), and the use of interfacial chemistry in nanoliters droplets for in vitro measurements of protein aggregation to understanding the amyloidosis biophysics was also proposed (Meier et al., 2009), however in the presented work micro and nanomaterials were used to perform a sensitive immunoassay for ApoE detection. First, the platform performance was evaluated by using a model protein, human IgG (HIgG) and finally the ApoE detection in plasma within a microfluidic platform, making the system easily scalable to a point of care (POC) system, was also demonstrated discussing the advantages and drawbacks of the microfluidic platform for real samples related to Alzheimer's diseases.

2. Materials and methods

2.1. Reagents

Real samples in plasma were provided by the research laboratory for Neurochemical Dementia Diagnostics, Department of Neurology, Ulm University, Germany. Human ApoE ELISA kit (HRP) was purchased from Mabtech (Nacka Strand, Sweden). Streptavidin – QD655 were obtained from Invitrogen (Spain). PBS, Tween 20, casein and bovine serum albumin (BSA) labeled with ALEXA 555 were acquired from Sigma Aldrich (Spain). PBS supplemented with 5% (w/v) of casein and 0.005% (v/v) of Tween 20 was prepared as blocking buffer. PBS supplemented with Tween 20 at 0.05% (v/v) was used as washing buffer (PBST). PBS was employed as immunobuffer, except for QDs conjugation. In this case, commercial borate buffer, at pH: 8.3, and 0.05% of sodium azide was used. Tosylactivated magnetic beads (2.8 μm of diameter) were also obtained from Sigma Aldrich (Spain) as well as the Human IgG, anti-Human IgG (α-HIgG), and biotin anti-Human IgG (biotin α-HIgG), both antibodies produced in goat.

2.2. Microfluidic device and screen printed electrodes fabrication

Microchips were fabricated by rapid prototyping and PDMS technology as described previously (Xia and Whitesides, 1998). Briefly, a 4 in. silicon wafer was spin coated with a negative photoresist (SU-8 from Microchem) and patterned by photolithography. PDMS was poured onto the resulting mold and cured at 65 °C for 4 h. The channel was 500 μm wide, 50 μm depth and 3 cm long.

The electrochemical detector consisted of a set of three electrodes of 500 μm width separated by 500 μm with an approximated thickness of 4 μm. They were produced by screen printing technology using a screen-printing machine (DEK 248, DEK International, Switzerland). Graphite ink (Electrodag 423SS) was used for the working (WE) and counter electrode (CE), and silver/silver chloride ink (Electrodag 6037SS) for the reference electrode (RE). All the inks were purchased from Acheson Industries, Germany. The fabrication process involved two steps. In the first step the graphite layer (WE, CE) was printed onto the PC sheet and cured at 80 °C for 40 min. The RE was obtained by printing a second layer of silver/silver chloride ink which was cured under the same conditions.

Finally, the PDMS channel and the PC substrate were assembled using a previously reported protocol (Tang and Lee, 2010). The PC substrate was treated by air-plasma for 1 min and then immersed into a 2% (v/v) 3-aminopropyltriethoxylane (APTES) (Sigma Aldrich) solution in water for 1 h. The surface of the PDMS channel was also activated for 1 min by plasma, and put into contact with the PC sheet to achieve irreversible bonding (Medina-Sánchez et al., 2012a, 2012b). Finally, a homemade connector was used for the electrical connection (see Fig. 1).

2.3. Characterization techniques

The morphology of streptavidin-QD655 was checked through high resolution transmission electron microscope (TEM). A 2 μL drop of QD solution (12.5 nM in milli-Q water) was deposited onto a holey carbon layer copper grid and then air-dried. A Tecnai TEM (USA) operating at 200 kV was used to obtain the images for posterior analysis. Magneto-immunoassay was also checked by MAGELLAN scanning electron microscopy (SEM), operating at 2 kV.

A Leica TCS SP5 AOBS spectral confocal microscope (Leica Microsystems, Germany) was employed to evaluate the effect of blocking step into the microfluidic channel.

2.4. Magneto-immunoassay in flow mode

A 40 μg tosylactivated magnetic particles were washed 3 times with borate buffer (200 μL) and suspended in 135 μL of the same buffer. 15 μL of capture antibody (α-HIgG or α-ApoE) at 0.5 mg mL^{-1} were added and the conjugation was achieved by gentle stirring of the suspension overnight at 700 rpm and 37 °C. The unbound antibodies were eliminated by washing 3 times with

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