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Integration of microcoils for on-chip immunosensors based on magnetic nanoparticles capture

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ABSTRACT

Immunoassays using magnetic nanoparticles (MNP) are generally performed under the control of permanent magnet close to the micro-tube of reaction. Using a magnet gives a powerful method for driving MNP but remains unreliable or insufficient for a fully integrated immunoassay on lab-on-chip. The aim of this study is to develop a novel lab-on-chip concept for high efficient immunoassays to detect ovalbumin (Biodefense model molecule) with microcoils employed for trapping MNP during the biofunctionalization steps. The objectives are essentially to optimize their efficiency for biological recognition by assuring a better bioactivity (antibodies-ovalbumin), and detect small concentrations of the targeted protein (~10 pg/mL). In this work, we studied the response of immunoassays complex function of ovalbumin concentration. The impact of MNP diameter in the biografting protocol was studied and permitted to choose a convenient MNP size for efficient biorecognition. We realized different immunoassays by controlling MNP in test tube and in microfluidic device using a permanent magnet. The comparison between these two experiments allows us to highlight an improvement of the limit of detection in microfluidic conditions by controlling MNP trapping with a magnet.

1. Introduction

Biosensors could be defined by the need to incorporate a biological, biologically derived or biomimetic recognition element with a transducing element [1]. In a simple way a biological response has to be detected by a tool and converted in one way or another into an electrical signal.

Since the first publication of Enzyme-Linked Immune-Sorbent Assay (ELISA) by R. S. Yalow in 1960 [2], the ELISA method has been used as a diagnostic tool in biology and medicine and it became one of the most specific method in immunoassay for Alzheimer diseases [3], HIV virus detection [4] or cancerous biomarker [5,6]. Particularly this technic allows a detection of low concentration. Conventionally ELISA method is performed in wells or tube. Three type of ELISA can be found direct ELISA, sandwich ELISA and competitive ELISA but a prepared surface is needed for all.

Another complementary method to ELISA has emerged using magnetic nano or micro beads [7] which are functionalized in fluidic conditions [8]. Since few years, the use of functionalized beads by trapping them in a magnetic field had opened the route to on-chip bead-based ELISA system. In fact, a higher sensitivity is obtained using micro-nano beads to increase surface to volume ratio combined with microfluidic devices [9,10].

Using MNP is a common way to realize immunoassay [11–14]. In this topic, MNP have really interesting properties such as (i) specific functionalization, (ii) easy manipulation, (iii) an important specific surface (surface/volume ratio) which offers us a better condition for biografting [15]. MNP are useful to realize an immunoassay on chip [12,16], by blocking them with magnetic field and injecting continuous flow with different solutions [9]. Another alternative consists on moving nano-beads through different solutions which permit to realize dynamic immunoassay [17].

Microfluidic devices are largely developed, in different domains, for instance (i) quantification [18,19] (ii) cell analysis [7,20]. Many functions such as sensing, mixing, manipulation [21–23], could be integrated on microfluidic devices with one aim to build a lab-on-chip environment. Microfluidic device can be made of PDMS (PolyDimethyl Siloxane). It is a silicone elastomer with desirable properties that make it attractive for microfluidic components and biomedical application. In fact, PDMS ensures thermal stability, permeability to gases, easiness for handling and manipulating [24]. These properties allow a rapid and low cost fabrication of microfluidic devices.

Nowadays ELISA using nanoparticles to detect pathogen bacteria [25] is extensively applied for food poisoning [26,27] since it was considered as a major health threat by governments and other applications are also pursued [28–30]. This specific ELISA method demonstrates that a very low limit of detection could be reached with a high level of sensitivity.

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The objective of this work is to integrate microcoils in a fluidic system in order to validate the concept of immunoassay formation and MNP trapping and detection by microcoils. In fact, in this paper we present one part of our work concerning the integration of immunoassay in microfluidic system which involves permanent magnet for MNP capture. The next step will be to implement microcoils of trapping and detection in a conveniently designed lab-on-chip.

2. Principle of magnetic trapping

MNP can be manipulated and controlled in fluidic device by a magnetic field created by a magnet or microcoils for instance [31–33].

As it known, when a particle is localized in a magnetic field $\overrightarrow{H_a}$, it obtains a magnetic moment aligned in the magnetic field direction:

$$\overrightarrow{M_p} = \chi \overrightarrow{H_a} \tag{1}$$

$\overrightarrow{M_p}$ as magnetic susceptibility and χ as magnetic moment per volume unit. We can distinguish two types of susceptibility: (i) intrinsic magnetic susceptibility χ_p which is the ratio of the magnetization to the applied field (ii) measured magnetic susceptibility χ_m which is the ratio of the magnetization to the local magnetic field inside the particle. Besides an equivalent bipolar moment $\overrightarrow{M_p}$ is usually obtained to calculate the magnetic force:

$$\overrightarrow{m_p} = \frac{3\chi_p(\chi_m+1)}{\left[\left(\chi_p-\chi_m\right)+3(\chi_m+1)\right]}\overrightarrow{H_a}. \tag{2}$$

Magnetic field creates magnetic force to control MNP. Magnetic force can be described as:

$$\overrightarrow{F_{mp}} = \left(\vec{m}.\vec{\nabla}\right).\vec{B} \tag{3}$$

$\overrightarrow{m}$ as the equivalent dipolar magnetic moment of each particles. When magnetic particles are suspended in a fluid, where μ_f is the permeability, the relation between $\vec{B}$ and $\overrightarrow{H_a}$ can be described as:

$$\vec{B} = \mu_f \overrightarrow{H_a} \tag{4}$$

If we assumed that the magnetization is linear, and according to Eqs. (2) and (4) the magnetic force acting on magnetic particle can be described as:

$$\overrightarrow{F_{mp}} = \mu_f V_p \frac{3\left(\chi_p-\chi_m\right)}{\left[\left(\chi_p-\chi_m\right)+3(\chi_m+1)\right]}\left(\overrightarrow{H_a}.\nabla\right)\overrightarrow{H_a} \tag{5}$$

V_p as the volume of a particle. We can extract from Eq. (5) two cases: $\chi_p > \chi_m$ where the particles will be under an attractive force and $\chi_p < \chi_m$ where the particles will be under a repulsive force. Magnetic force can be used to attract and repulse magnetic particles and the strong of the force depend of several parameters including magnetic field and parameters of the particle.

Regarding this consideration, simulations have been performed on ANSYS® software to define the convenient design of microcoils for efficient MNP trapping. Indeed, in this paper we present microcoils with the following optimized parameters: 45 turns, width of Copper wires = 10 μm, distance between wires = 10 μm, height of wire = 10 μm, outer radius of the coil = 5 mm (Fig. 3).

3. Principle of immunoassay sandwich involving magnetic nanoparticles

The enzyme-linked immunosorbent assay (ELISA) is a test that uses antibodies to detect the presence of a substance (target), usually a biomarker, in a liquid sample or wet sample.

Ovalbumin is used as a model molecule for biodefense and as target for the developed ELISA protocol in this work. The principle is to encapsulate the target (a biomarker) between two antibodies and one antibody for the detection. Primary antibody recognises a part (epitope) of the biomarker, the second antibody recognises another epitope of the biomarker. The detection antibody recognises a part of the second antibody and the resulting structure forms the biological complex (immunoassay sandwich, Fig. 1b). This complex is completed by a magnetic nanoparticle where the primary antibody is grafted with an EDC/NHS protocol at 4 °C, Fig. 1a.

In addition, BSA is used to avoid the non-specific absorption of biological elements, this step is performed after the primary antibodies grafting to fill up free surface between antibodies. As it shows in Fig. 1c, a monolayer of antibodies could be grafted on one magnetic nanoparticle therefore several biological complex could be observed.

4. Material and methods

4.1. Material and experimental equipment

A microfluidic platform is used to perform immunoassay according an ELISA protocol. A fluidic pump and a syringe are used to inject fluid inside the microfluidic system. A Peltier module with an electrical equipment and a temperature sensor are used to regulate the temperature during experiments. A fluorescent microscope (Euromex Série B) with 4x NeoLED™ for monochromatic LED Epi-Fluorescence and standard filters (Green EX 460–550 nm EM 590 nm) is used to visualize fluorescent antibodies.

The microfabrication of microfluidic devices and microcoils are made in a clean room. Two types of 4 in. wafers are used, silicon wafer and glass wafer.

Different equipment are used for microfabrication: UV-photolithography EVG 620 for print panel on photoresist. Electrolyte bath using Cu_2SO_4 and H_2SO_4 with integrated electrode of copper for electrodeposition of copper. Denton sputtering system (EXPLORER Denton Vacuum® system) and PECVD (STS PECVD System) for depositing layer. Reactive Ion Etching (STS RIE system) and Ion Beam Etching (IBE Roth & Rau IonSys 500) for etching layer. DEKTAK 8 (Veeco) and Keyence microscope for characterization.

MNP with carboxylic terminal group are purchased from Adamtech © (Carboxyl-Adembeads and MasterBeads Carboxylic Acid) and are cleaned with NaOH 1 mM solution for one day.

Biological and chemical material are used in ELISA protocol: EDC/NHS (*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide/*N*-hydroxysulfosuccinimide from Sigma-Aldrich ©) for grafting. Primary antibodies: Rabbit Anti-Ovalbumin (Hen White) from Merck Millipore ©. BSA (Bovine Serum Albumin) from Sigma-Aldrich © for passivation. Ovalbumin (Albumin from chicken egg white) from Sigma-Aldrich ©. Secondary antibodies: Purified anti-chicken ovalbumin from BioLegend ©. Detection antibodies: Anti-mouse IgG (Fab specific)-FITC antibody produced in goat from Sigma-Aldrich ©. PEO solution (Poly(ethylene oxide) from Sigma-Aldrich ©).

4.2. Microfabrication

4.2.1. Microfluidic on glass wafer

Microfluidic devices are composed of two parts: glass wafer with a thin layer of PDMS (20 μm) spin-coated on it and fluidic channels made of PDMS. A schematic illustration of the process is presented in Fig. 2a.

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