Détection de nanoparticules magnétiques dans des dispositifs microfluidiques hybrides à base de GMI et de microbobines pour le diagnostic biomédical

« NANOBIOGMI »

Participants :

IEF (UPSUD UMR 8622) : Meritxell Cortès (Post-Doc), Hong Ha Cao (Doctorant), Mehdi Ammar (MCF), Johan Moulin (MCF-HDR), Marion Woytasik (MCF), Emile Martincic (MCF), Elisabeth Dufour-Gergam (PR)

LGEP (UPSUD UMR 8507) : Yann Le-Bihan (PR)

SATIE (ENS CACHAN UMR 8029): Frederic Mazaleyrat (PR)

Context:

The project aims to develop bio-microsystems based on a highly sensitive magnetic detection using a micro-sensor network composed of magneto-impedance (MI) and/or microcoils. Achieving this goal requires the use of suitable biocompatible particles for the magnetic contrast. In addition, it is necessary to develop highly sensitive systems for the specific detection of DC magnetic fields and/or alternative low intensities resulting from interaction between the nanoparticles and the applied field. The device will associate the sensor matrix and a microfluidic circuit (lab-on-chip) with a conveying system of nanoparticles based on the gradient of magnetic field applied by the integrated microcoils ("moving and trapping of nanoparticles"). This will constitute the first proof of concept of a sensing device. Consideration will be given subsequently to functionalize the nanoparticles and the inner surface of the sensor by biological entities.

A) Results on electromagnetic simulation

- Modeling for magnetic manipulation of nanobeads using microcoils embedded in PDMS

The simulation work was performed with FE software (Ansys® academic research 12.1). FE calculated magnetic flux density generated by the coil (square, 90 turns), I=1A extracted along the red dotted line.

Figure 1. (left) overview of the region where magnetic field was simulated (right) flux density function of vertical distance representing the thickness which separate the channel to the microcoil plane.
The most interesting result that we could highlight by this simulation work concerns the $|\mathbf{B}|$ gradient which is higher at the edges of the coil. In addition, PDMS protection layer of copper coil needs to be as thin as possible (< 50µm).

**B) Results on GMI sensors**

MI sensors with different width, length and thickness were prepared. The sensors consist in three stacked layers of different metals, two permalloy layers with a copper one in the middle, and also two copper contact pads at both sides. The fabrication difficulties lie in that sometimes some of the layers do not exhibit good adherence. Sensors of 5 mm long with a permalloy width ranging from 50-250 nm and a copper one from 10-100 nm have been performed.

**Figure 2.** (up) Optical image of a general view of the MI sensor and (down) SEM image of a transversal section of the sensors.

The sensors were annealed at both 300° C and 500° C, in some cases, a magnetic field was applied at the same time that the annealing was performed.

Measurements were performed by measuring the resonance voltage generated by the (R,L) microsensor and an added capacitor. The sensors were excited at different frequencies in order to find the optimum one, while the supplied excitation current was fixed at 1 mA by using a 1 kΩ resistance in series. An external longitudinal DC magnetic field was created by a solenoid, which was controlled by a DC power supply. Finally the voltage measured at the terminals of the sensor, which is modulated by the DC field, was demodulated by a lock-in amplifier.

Results showed that the best sensibility was achieved when the sensors were excited at the resonance frequency ($F_r$). The post annealing process decreases the sensibility. In the case of comparing different thick sensors, the best results were obtained for the ones with the thinnest copper layer, reaching sensibilities of 13 kΩ/T.
Figure 3. Relative voltage with the magnetic field for A) the same sensor at different frequencies B) for different thickness sensors at the resonance frequency (Fr) Cu thickness: S1>S2=S3>S4, PM thickness: S1=S3>S2=S4.

As the final objective of the project is the integration of the MI sensors with microchannels to fabricate a microfluidics chip, a new mask for the microchannels preparation was performed. Microchannels consist in two layers of SU-8 covered with a thick piece of PDMS. The first layer of SU-8 (5 µm) was deposited in order to isolate the sensors for, the fluid and only the holes for the contacts were left. The second layer of SU-8 (50 µm) was patterned with the holes for the contacts and the 50 µm thick micro channel. In order to stuck the SU-8 and the PDMS a silanization was successfully performed.

![Silanization process](image)

Figure 4. (Left) Schematic representation of the microchannels fabrication process (right) Microchannel image.

The fluidic tests and the first measurements of magnetic nanobeads injected in the fluidic channels are in progress.

C) Results on magnetic manipulation of nanobeads using embedded microcoils

![Microfluidic chip with integrated planar spiral Cu microcoils](image)

Figure 5. Microfluidic chip with integrated planar spiral Cu microcoils.

The aim of this part of the project is to enable (i) trapping magnetic nanoparticles using integrated microcoils, (ii) Functionalizing surface of nanoparticles inside channel; (iii) pre-concentrating bio-marker coupled with function group on surface of nanoparticle (iv) carrying out bio-analysis processes.
1) **Process of microfabrication of the fluidic microchip**:

**Process of microcoil fabrication**

1. **Fabrication of PDMS channel**
   - PDMS mold Fabrication by SU-8 on Silicon wafer:
   - PDMS casting: Casting, curing PDMS and Peeling PDMS out of SU-8 mold

2. **Fabrication of copper coil**
   - Step 1: Metallic seed layer depositing on Si wafer
   - Step 2: Photoresist spin-coating
   - Step 3: UV-exposure with photomask
   - Step 4: Copper electrodeposition
   - Step 5: Photoresist removing
   - Step 6: Seed layer etching

3. **Assembling microfluidic chip**
   - PDMS channel with Nanoports
   - Copper coil on Si wafer.

**The first microfluidic chip**

- Square coil (1.2x1.2 cm): 90 turns, Cu wire: 10 µm, 12 µm high; space: 10 µm.

2) **Thermal characterization**:

The injected current provokes an increase of the coils’ temperature by Joule effect. A thermocouple is placed inside the PDMS channel to measure the temperature on surface of copper wire. The influence of applied current on temperature in channel was characterized in two experiments: one with the injection of a liquid by pumping and the other without injecting any fluid (see figure 6).

![Figure 6](image)

Figure 6. (left) microchip integrating a couple of microcoils crossed by a PDMS microchannel (right) the thermal characterization of the microchannel.

To work with temperature under 40°C (compatible with biological applications) the current has to be lower than 60 mA (or current density < 5 x 10^8 A/m²), as we use parameters of this microcoils. The presence of liquid flow inside channel does not affect the temperature of microcoil surface.

3) **Magnetic nanoparticles trapping**:

Magnetic nanoparticles (NPs) are trapped by microcoils applying a current of \( I = 120 \ mA \) (\( i = 6.7 \times 10^9 \ A/m^2 \)), \( T^o = 40^\circ C \) on surface of Cu wire, (83 turns; Cu wire: 15 µm wide and 12 µm high, track space: 10 µm) and PDMS protection layer was fixed to 10 µm. We can observe
three steps of the trapping mechanism in the figure 7: (1) Dynabeads® MyOne™ Carboxylic Acid (1.05 µm) are dispersed in water and injected in channel with a flow rate of 0.049 cc/hr (or 0.82 µL/min) (2) The NPs trapping occurs at the Cu wire, which generate the highest magnetic field as simulation result shown, when microcoil is applied a current in about 1 minute (3) NPs are released after cutting off current.

**Figure 7.** Image focused on the inner border of the microcoil showing the trapping of the nanobeads when the current is switched on.

D) Immune-capture for detecting biomarkers of Alzheimer disease (Aβ 1-42) (in collaboration with Institut Galien Paris-Sud, Laboratoire Protéines et Nanotechnologies en Sciences Séparatives)

**Figure 8.** (a) immune-reaction between immobilized mouse IgG and anti-fab Hrp labeled (b) Sandwich immunoassay with a capture antibody, the antigen (Amyloid peptide Aβ 1-42), the recognition antibody and the detection antibody labeled with Cy5.

**Figure 9.** Fluorescent microscopy analyses showing the sandwich immunoassay performed as depicted in the figure 8, with (a) and without (b) the presence of capture antibodies on the surface (1st layer).
Before integrating magnetic nanobeads and manipulating or detecting them in fluidic chip, we need to experiment immune-sensing techniques which are compatible with the use of magnetic nanobeads. In fact, an immune-sensing platform was applied to one Alzheimer disease biomarker, the amyloid peptide, Aβ 1-42. The whole sandwich immunoassay was performed following the procedure depicted in figure 8-b. After immobilizing the capture antibody directed against Aβ 1-42. The standard amyloid peptide was allowed to react with the capture antibody. After washing, a second antibody directed against amyloid peptide Aβ 1-42 was allowed to react with the bound biomarker. Then, a third recognition antibody targeting mouse Antibody labeled with Cy5 was added. After washing procedure, the surfaces were observed by fluorescent microscopy. The linearity of the response was obtained in a concentration range from 2.5 to 7.5 µg/mL. The determination coefficients 0.988 showed an excellent linearity compatible with a fully quantitative method. The limit of detection (LOD), chosen as the concentration corresponding to the background signal plus three times its SD, was estimated at 300 ng/mL (66 nM) . This value is closed to the level of total Aβ peptides found in the cerebrospinal fluid (CSF) (around 1 nM). However, it should be noted that this peptide, Aβ 1-42, has propensity to form oligomers even in in-vitro environment which can lead thereby to a decrease of its capture efficiency and thus to its detection sensitivity. Interestingly, the overall results show that the implemented immune-sensing platform coupled to fluorescent detection presents a valuable tool for pathologies diagnosis and could be consequently applied to magnetic detection. In fact, from the presented work we are trying to replace the fluorescent dyes (Cy5) by magnetic nanobeads and validate (i) all steps of biofunctionalization through microchannels by manipulating the beads (ii) demonstrate an ultra-sensitive magnetic detection using MI sensors or microcoils (work is in progress).

**Highlights**

Following the results of the first year of the project, several significant events (and perspectives) should be precised:

- Oral presentation scheduled on the 24th Anniversary World Congress on Biosensors, Melbourne, 26-30 May 2014 in Melbourne, [http://www.biosensors-congress.elsevier.com/congress-program.html](http://www.biosensors-congress.elsevier.com/congress-program.html);
- Acceptance of the project MicroDynaFonc «Fonctionnalisation dynamique de surface en conditions microfluidiques» (« projet Lasips 2ème appel émergence », 2014-2017);
- Acceptance of the project AMMIB "Analyse MultiModale Intégrée pour la Biodéfense" (ANR-DGA - programme ASTRID 2014-2017), 5 partners with IEF as coordinator.

**References**

**Articles**


**International conference**


M. Cortès, T. Peng, M. Woytasik, J. Moulin; «NiFe-based MI sensors micromolding» Accepted for 10th European conference on magnetic sensors and actuators (EMSA). Vienna (Austria) 6-9 July, 2014 (poster communication)

**Scientific meeting**