The rapid detection and quantification of one or more biological agents has become essential to anticipate a possible public health threat (epidemic or pandemic), environmental or other contextual threats. In this area, one of the main objectives is to facilitate this detection with a portable, rapid, cost-effective, sensitive and reliable lab-on-chip system. To achieve this goal, it is therefore necessary to design and build microsystems composed of innovative sensors. The biological analysis based on high sensitivity magnetic measurements is a new type of immunological diagnosis using magnetic nanoparticles (MNP) as markers instead of the enzymes, radioisotopes or fluorochromes conventionally used. This new method of analysis involves the coupling of antibodies or antigen proteins to MNP. In this project, the specific binding of the antibody to its antigen will be assessed by the detection of MNP of various sizes and also different magnetic properties through a planar micro-sensor that measures the induced magnetic field variation. The signals measured by the magnetic gradiometer are functions of the quantities of immune complexes (antibodies + analytes). The microfluidic channels integrated in this system make it possible to use very small quantities of reagents (microliters), thus reducing by several orders of magnitude the volume of samples to be used. The time required for bioanalysis will also be considerably reduced which can be very significant in the case of epidemics or pandemics, water, food and environmental security. This PhD thesis is based on the complementary and multidisciplinary skills of the advisors and also of different research teams collaborating to this project at Sorbonne University (GeePs, CIMI, LISE and PHENIX) and in Germany (Institute of Bioelectronics, FZI Juelich). In the continuity of the preliminary works on a microsystem with single pathogen detection, the objective of this PhD is to carry out the design, the multiphysics modeling and simulations, the fabrication, the characterizations and the validation of a portable microsystem associated with microfluidic reservoirs for multipathogenic detection of pathogens such as SARS-CoV-2 in biological fluids (saliva, urine, blood).
Subject

Context:

The detection and quantification of one or more biological agents has become essential to anticipate a possible health threat (epidemic as Ebola and Zika or pandemic as COVID-19), environmental or other contextual threats (bioterrorism, chemical and biological weapons). In this area, one of the main objectives is to facilitate this detection with a sensitive, reliable, portable and low cost lab-on-chip system.

The present COVID-19 pandemics caused by the SARS-Cov-2 virus illustrates how important it is to develop sensitive diagnostic assays that could be widely used in the population, preferably avoiding the need for blood samples. Saliva testing would be particularly convenient, but also medically-relevant, as COVID-19 is mainly a respiratory condition primarily inducing mucosal (IgA mediated) immunity.

The biological analysis based on high sensitivity magnetic measurements is a new type of immunological diagnosis using magnetic nanoparticles (MNP) as markers [1-8]. This new method of analysis involves the coupling of antibodies or antigen proteins to MNP. The specific binding of the antibody to its antigen will be assessed by the detection of MNP of various sizes and also different magnetic properties. Thanks to their extractability and sortability, MNP are suitable for the examination of biological samples, serving as markers for biochemical reactions [2, 7, 8].

Up to now, the final detection step is mainly carried out by the ELISA colorimetric method, fluorescence-based techniques or markings with radioelements. The standard enzymatic detection used in the ELISA method has a limited sensitivity (approximately 100 ng/ml) and a relatively long measurement time (approximately 10 min). This method as well as the fluorescent methods have a limited dynamic range and require the use of transparent materials and non-colored and non-fluorescent media. The use of radioactive markers is also problematic because of the regulations on radiation protection. Therefore, the immunoassays which detect the analyte by means of MNP constitute a very promising alternative with a sensitivity already obtained of 6ng/ml in a macroscopic system (10x23x7 cm3) using coaxial cylindrical coils and an ABICAP reservoir for the sample [2, 6-8].

The MNP covered on their surface with a biocompatible streptavidin layer can be specifically linked to analytes (proteins, viruses or bacteria), figure 1. Among the available magnetic techniques, the new technique based on the principle of frequency mixing has a definite advantage by making possible to quantify MNP with a very broad dynamic [8]. By studying the characteristics of the response signal (amplitude and phase) at a well-defined frequency, non-linear and specific signatures of different types of MNP can be discriminated.
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For this research project, contacts and collaboration have been established between GeePs, CIMI and other SU laboratories (LISE and PHENIX) and the "Institute of Bioelectronics" in Juelich (Germany). A miniaturized structure of planar and multilayer microcoils associated with a suitable microfluidic structure has been designed and realized (Figure 2, see in references the French-German patent issued in 2019). A bench of magnetic measurements, with frequency mixing and synchronous detection techniques coupled to a microfluidic bench was also set up [1, 3-5].

Besides the team led by Guy GOROCHOV at CIMI has a great expertise in the field of immune / microbe interactions mediated by IgG and IgA antibodies [11-13]. The GOROCHOV team also has the ability [14] to engineer human recombinant anti- SARS-Cov2 antibodies that could be used within the framework of this program in order to capture SARS-Cov2 antigens. He is supported by ANR and Sorbonne University by iCOVID program. This joint PhD project between CIMI and GeePs fits in perfectly with the Alliance Sorbonne University's scientific policy to support activities of the SU institutes and initiatives for the development of interdisciplinary biomedical research. This project combines the complementary scientific and medical skills and knowledge of the involved teams [1-14] on an important international public health issue with high sanitary, economic and social impacts.

Objectives:

The ultimate goal of this medical and engineering PhD project is to perform portable magnetic immunoassay and multipathogen sensing on a chip. Detection of pathogens such as SARS-Cov-2 in human samples, mainly saliva but also urine and blood will be achieved using the experimental knowledge and facilities at CIMI. A microsystem using MNP markers in a microfluidic channel as microliter sample holder will be developed. Fully integrated planar excitation and sensing microcoils will be designed and fabricated on both sides of a suitable microfluidic chip with multiple micro-analysis reservoirs with optimized size and shape. Analytical and numerical multiphysics modeling and simulations (Electromagnetic, thermal, microfluidic and biochemical reaction) of the magnetic detection and actuation will make possible to optimize the parameters of the integrated microcoils. These models will also allow to evaluate the performances of the microsystem in terms of sensitivity and specificity.

The MNP will be synthesized by PHENIX to get mono-core, multi-core and core-shell nanoparticles with different sizes and magnetic properties. They will be compared also with other commercially available MNP in innovative microfluidic structures in collaboration with LISE laboratory. These MNP will be characterized according to their non-linear magnetic responses. They will be surface-coated and functionalized with so-called "detection" antibodies or antigen proteins for the specific detection of the analyte in collaboration with CIMI. The surface of the fluidic channel in the microsystem will be functionalized by grafting with appropriate capture biological entities in collaboration with CIMI. The magnetic actuation will be tested as an analyte detection trapping technique based on magnetic relaxation time measurements. Compared to current techniques, the operational speed of this new method will allow access to real-time measurements. C-Reactive Protein (CRP) will serve as the first biological proofing entity before extending the application to other specific and relevant biochemical entities such as procalcitonin (PCT) and SARS-Cov-2 spike antigen for human or animal medical diagnosis.

Expected results:

This PhD thesis consolidates activities of GeePs and CIMI in innovative bio-testing methods and microsystems for immunological applications. A real-time measurement of the concentration of viruses such as SARS-Cov-2, bacteria and proteins and a test of their functionality based on the interaction of the antigen-antibody becomes possible. The results will have high public health impacts with a portable, fast, ergonomic and cost-effective multipathogens detection system and lead to technological transfers to companies, which develop Point-of-Care (POC) biological analysis systems.
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References:


French-German European patent: