Surface plasmon resonance sensor technology for early detection of biomarker proteins in whole blood, in point of need settings (MarkerSense)

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ABSTRACT

Blood biomarkers are currently analysed in specialised laboratories by expensive and time-consuming procedures, however unsuitable for life threatening situations, where early detection is vital (*e.g.* sepsis). To address this challenge, we developed a novel point-of-care system for fast and sensitive detection of biomarkers in whole blood, enabling early diagnostics (MarkerSense). Based on a proprietary Surface Plasmon Resonance technology, developed by project Coordinator, coupled to a smartphone, MarkerSense simultaneously performs high signal-to-noise ratio reflectivity and phase angle resolved assays, in a portable format. After validation in clinical trials, MarkerSense is envisaged to be used for rapid diagnosis of sepsis in hospitals.

Keywords: surface plasmon resonance; point-of-care; sepsis.

1. INTRODUCTION

- Detection of disease biomarkers directly from patient samples, enabling rapid and direct feedback to healthcare providers is essential in life threatening situations, such as the risk of sepsis and septic shock or current SARS-Cov-2 pandemics;
- The project breakthrough technology enables high signal-to-noise ratio reflectivity and phase angle resolved Surface Plasmon Resonance assays in a compact, point of care (POC) portable format for fast (minutes) and sensitive detection of biomarkers in a small volume (~100 µl) of whole blood;
- MarkerSense main results are: 1) prototyping and integration of Coordinator's (ICB) proprietary technology to provide the angle resolved curves of both reflectivity and phase in a hand-held, smartphone operated, compact Surface Plasmon Resonance (SPR) system for ultra-sensitive analyte detection; 2) a solid state refractive index matching surface for optical coupling of sensing components for hand held operation; 3) a user-friendly acquisition and analysis software to drive the complete assay; 4) a microfluidics chamber developed by photolithography to deliver the sample (blood) to the sensing interface; 5) the sensing chip, *i.e.* a novel plasmonic functional thin film with biorecognition add-layer, optimized for high sensitivity and reduced non-specific binding.

2. STATE OF THE ART

Early detection of blood biomarkers could mean the difference between life and death in life threatening diseases, such as sepsis and septic shock. Current clinical and laboratory measurement techniques use time consuming procedures to filter the blood cells and then measure proteins present in very low concentrations[1] with labelled reactant methods (such as ELISA, RIA etc.), delivering the results within at least 24h. The equipment is also bulky, preventing the miniaturization in a point-of-care format. Therefore, a POC, portable, fast and sensitive device that can be easily used to detect and quantify in minutes a large variety of disease biomarkers, and providing direct feedback to physicians and/or nurses in hospitals, emergency departments, home care, etc., brings major advancements to the world of *in vitro* diagnostics.

SPR based assays were successfully used for biomarkers detection in plasma and serum[2]. However, traditional SPR devices generally require expensive equipment, complicated optics, and precise alignment of the components, features that hinder the development of portable devices. Lately, emergence of smartphones with internet connectivity, high-resolution cameras, and high-performance CPUs has facilitated the development of SPR based POC devices[3], however, with relatively high limits of detection (~50 nM) and low resolutions (~7.4 x

10^{-5} refractive index units (RIU)), therefore being unsuitable for evaluating biomarkers present in low concentrations. This is typical when monitoring the shift of SPR dip or intensity under a fixed incidence angle/wavelength, where a minimum resolution of 10^{-7} RIU was achieved[4]. A different approach, based on SPR phase detection, provided a resolution of 10^{-8} RIU or better[5]. Moreover, phase noise is a few orders lower than intensity noise. However, an inherent drawback for phase SPR sensors is their limited dynamic range. Hence, a design combining phase detection and angular interrogation is expected to provide both high sensitivity and wide dynamic range[6].

3. BREAKTHROUGH CHARACTER OF THE PROJECT

As an alternative to existing technologies, the project developed a portable, fast, sensitive, phase and reflectivity angle resolved SPR system, with integrated signal acquisition and analysis, illumination and sample delivery. The system, named MarkerSense, is based on a proprietary technology of the Coordinator advancing a compact SPR optical device coupled to a smartphone to detect low concentrations of biomarkers (*e.g.* proteins) in whole blood in a few minutes. As a novelty element, in addition to the phase information, the SPR system simultaneously provides the conventional SPR dip, for an increased signal-to-noise ratio. The sensor is illuminated by the phone LED flash coupled on an optical fiber, while the reflected light is detected by the phone camera. A regular smartphone is integrated via a SPR adaptor that can be conveniently installed or removed. Besides providing the light source and camera, via a custom application, the smartphone is used to derive the reflectivity and phase information from the acquired camera images (Fig. 1) and display the target results (based on calibration curves). The project benefited from Coordinator (ICB) expertise in SPR instrumentation[7], data acquisition systems and computing[7–9] and SPR measurements of blood proteins in complex media[10]. Also, the project benefited from Partner 2 (IOEL) expertise in developing custom optical components and instrumentation, who developed the optical component required for simultaneous phase and reflectivity measurements and the 3D-printed custom support for all parts, including the smartphone. Another important feature of the MarkerSense is that the measurements are performed in whole blood, requiring a small volume (~100 µl). This eliminates the centrifugation step, reducing the assay duration to a few minutes. Since SPR is sensitive only to binding events taking place in the immediate vicinity of the sensor surface (~200 nm)[11], the use of a thick hydrophilic matrix (~500 nm) both ensures immobilization of the recognition elements and protects the surface from blood cells adhering to it.

4. PROJECT RESULTS

Current status of the project relates to the following accomplishments:

1. A hand-held compact SPR system to provide the angle resolved curves of both reflectivity and phase to boost the sensitivity of analyte detection (Fig. 1).
Basically, the system uses the fibre coupled light provided by the smartphone flash LED, filters it (around 780nm) and projects it at a set of angles of incidence, centred on an angular value corresponding to the resonance angle. The underlying proprietary technology[12] based on a plan parabolic lens and a couple of reflective surfaces couples the incident light into the thin metal film that covers the surface of tailored glass substrate chips. Moreover, a custom polarizer with 3 polarizing areas (0°, 45° and 90°) was developed to provide the 3 desired polarization states for phase derivation. The reflected beam then passes through an analyser oriented at 45° and is focused on the smartphone camera. This allows common path, simultaneous measurements for 3 different polarizations. For each polarization, the intensity curve versus incidence angle is calculated by averaging the corresponding pixel intensities (I_0, I_{45} and I_{90}). Moreover, this method allows self-referencing for each acquisition.
The SPR phase (φ) is derived for each incidence angle according to the equation (1):

$$I_{45} = I_0 + I_{90} + 2\sqrt{I_0 I_{90}}\cos\varphi \qquad (1)$$

Therefore, as a novelty element, the reflectivity and phase curves versus incidence angle are simultaneously derived in a single measurement. This increases the signal-to-noise ratio, allowing very sensitive measurements of the target analytes.

2. A solid state refractive index matching surface for optical coupling of sensing components.
Typically, the optical coupling is achieved by using refractive index matching oils or gels. The refractive index matching material is placed between the back of the gold-coated sensor and the prism surface. However, the low viscosity of the optical oils can cause contamination of the sensing surfaces, rendering this simple procedure unsuitable for portable and field-deployable instruments, where simplicity and rapidity are the main requirements[13]. To address these drawbacks, a solid-state refractive index matching surface for optimum optical coupling of the sensor chip to the plan parabolic lens was developed. Based on a polymer with refractive index similar to that of the BK7 glass, the solid-state optical coupling solution had similar performances with the refractive index matching oil.

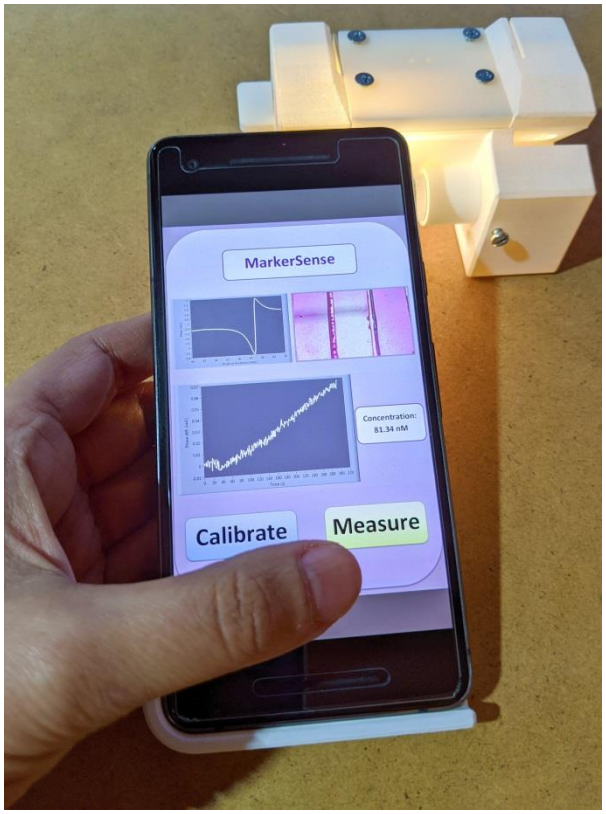

Fig. 1. The portable and compact SPR system MarkerSense for early detection of biomarkers in point of need settings

3. A user-friendly acquisition and analysis software to drive the complete assay (*i.e.* set camera exposure time, control the LED flash light, acquire images, derive the relevant parameters and plot the results).
Dedicated software was developed for Android and iOS smartphones. First, the software sets the camera exposure time and controls the LED flash light. Then, it acquires images and derives the SPR minimum and phase values. The results are plotted on a graph in real-time and the achieved level is transformed in concentration, based on a previously derived calibration curve (Fig. 1).

4. A microfluidics chamber developed by photolithography to deliver the sample (blood) to the sensing interface.
A polydimethylsiloxane (PDMS) microfluidic chamber, with a total volume of ~100 µl was developed by photolithography. The chamber is placed on top of the sensor chip, is secured by 4 screws and delivers the sample to the sensing surface by capillarity (Fig. 1).

5. The sensing chip, *i.e.* a novel plasmonic functional thin film with biorecognition add-layer, optimized for high sensitivity and reduced non-specific binding.
The sensor gold surface was functionalized with a thick carboxymethyl-dextran matrix (~500 nm) to keep the blood cells outside of the sensing region and prevent them from interfering with the measurements. Additionally, the hydrophilic dextran matrix has the role of reducing nonspecific binding of other molecules present in blood. This solution has been also successfully applied by the Project coordinator to detect thrombin in serum[10]. The functionalization layer was tested for nonspecific binding and cells interference, and the limit of detection for a sepsis marker *i.e.* soluble CD25 (sCD25), Fig.2, was ~2 ng/ml, as compared to relevant concentrations[14] for early diagnosis of sepsis (~3 ng/ml).

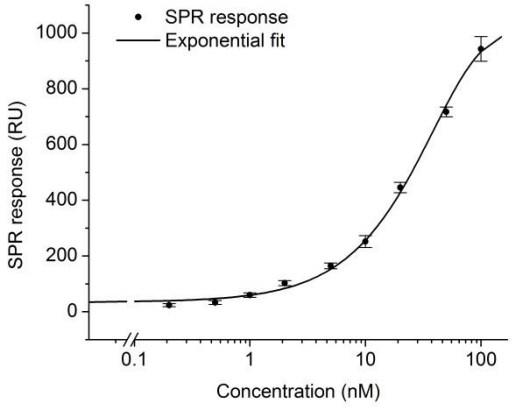

Fig. 2. Calibration curve for sCD25 obtained with the sensing chip

5. FUTURE PROJECT VISION

5.1. Technology Scaling

It is envisioned that during ATTRACT Phase 2, MarkerSense will start from Technology Readiness Level, TRL 4 (technology validated in lab), and will achieve TRL 7 (system prototype demonstration in operational environment), as a POC device.

For this, we plan to participate in a clinical trial for assessing early diagnostic of sepsis. The trial will involve detection of sepsis performed by both standard clinical assays and by MarkerSense to assess sepsis biomarkers by direct analysis of whole blood samples.

5.2. Project Synergies and Outreach

Since our product is based on 3D printing technology and the use of a smartphone, it is suitable for affordable mass production in low to medium quantity, with minor modifications and process optimization. A large cost reduction could come from 3D printing of the optical components.

For manufacturing the compact optical setup for the portable SPR system, a partner specialized in industrial 3D printing will be necessary. Also, a partnership with a hospital will speed up the calibration, optimization and validation steps.

5.3. Technology application and demonstration cases

During the ATTRACT Phase 2, we plan to implement a technology demonstration case that will bring concrete benefits to the areas of Scientific Research, Industry and Societal Challenges, more specifically in the Health domain.

Project value will be confirmed by rapid (minutes) detection of sepsis biomarkers (including the type/s of the causing microorganism/s: bacterial/fungal/viral), directly from blood. Applications aim for early diagnostic of life-threatening infectious diseases e.g. sepsis, along with other diseases with known biomarkers in blood/biofluids. Also, the envisaged link with a system allowing pathogen detection & phenotypic antimicrobial testing (under development within ICB) will support ground breaking POC fast diagnostic *i.e.* microbe identification and prescription of the effective antibiotic.

Early identification of patients who are at high risk of death and who might benefit most from early and aggressive treatment represent a critical step in sepsis management. Therefore, on a short term (year 1-2), the POC device developed by the project is envisaged to participate in a clinical trial for assessing early diagnostic of sepsis. On medium term (year 2-5), following successful clinical trials, MarkerSense is envisaged to be used for rapid on-site detection of sepsis biomarkers in hospitals (emergency departments). On long term (year 6-10), MarkerSense will also provide fast identification of widespread diseases such as COVID-19, pneumonia, hepatitis A, HIV, malaria or tuberculosis. Significantly, the technology will open a window of hope for the economically disadvantaged countries and low-resource environments, where most of the population does not have access to hospitals or clinical laboratories.

5.4. Technology commercialization

We plan to participate to science fairs and brokerage events dedicated to lab on a chip/POC bio-medical applications to identify potential investors and present/demonstrate the virtues of our system. Also, we intend to contact world class Diagnostic Companies and manufacturers of medical equipment to discuss collaborative routes for reaching the market including a partnership for system testing optimization as well as licensing.

Since our approach is ground-breaking, we plan to apply for private and/or public funding to advance our product, making it ready to go to the market.

The required budget for the Phase 2 MarkerSense project is estimated at 550 000 Eur.

5.5. Envisioned risks

The core risks (with their corresponding mitigation strategies) that our project will face in a potential ATTRACT Phase 2 project are as follows:

- 3D printed optical components provided by commercial alternatives are of inadequate quality – we will identify and initiate collaborations with research groups involved in 3D printing of optical components;
- High costs related to analysis consumables – we will investigate the use of aptamers and Molecularly imprinted polymers, MIPs, as low-cost alternatives to antibodies for the detection of biomarkers;
- The smartphone software is not user-friendly enough – based on the feed-back provided by the physicians and/or nurses during the clinical trial, we will improve the user experience by optimizing the software accordingly.

5.6. Liaison with Student Teams and Socio-Economic Study

During the ATTRACT Phase 2, collaborations with MSc. level student teams are foreseen for developing novel ideas and prototypes inspired by the project to address Societal Challenges. For this, an experienced person will be nominated to facilitate MSc. level explanation materials of our technology and to coordinate brainstorming meetings with the MSc. level student teams. A dedicated module coordinated by ICB within the Biomedical Master Programme at the Polytechnic University has already started in 2019 and is set to continue.

Also, during the ATTRACT Phase 2 we will contribute to the expert-driven socio-economic study of the ATTRACT initiative and ecosystem, by performing interviews, providing technology impact references, etc.

6. ACKNOWLEDGEMENT

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