Optical Biosensing Universal System - OBUS

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ABSTRACT

Biosensors and in particular optical sensors, where it is focused the current project, is a growing market with an expected annual increase of 9% within the next decade. The utilization of fiber optic for biosensing applications presents a unique opportunity thanks to the inherent advantages, such as small size, light weight or biocompatibility among others. In particular, high-sensitive lossy mode resonance (LMR) based optical fiber sensing devices present a unique opportunity to explore the detection of microRNAs (miRNA) associated to rheumatic diseases by means of the selection of the adequate biomarkers panel as it is described in this document.

Keywords: Rheumatoid Arthritis, optical biosensor, lossy mode resonances, biomarkers, miRNA

1. INTRODUCTION

Optical biosensors are a growing market with an expected annual increase of 9% within the next decade. In particular, the utilization of fiber-optic for biosensing applications presents a unique opportunity thanks to the inherent advantages, such as small size, light weight or biocompatibility among others. Currently, there are an ample range of fiber-optic interrogation techniques suitable to be used in biosensing applications, such as fluorescence, interferometry or surface plasmon resonance, which have been exploited by different multinational companies (GE Healthcare, Reicher, Horiba Jobin Yvon, ICx Nomadics, Bio-Rad Laboratories or Fortebio) with equipments valued from tens to hundreds of euros.

Particularly, fiber-optic lossy mode resonance (LMR)-based devices have been proven as devices with one of the highest sensitivities for refractometric applications [1-3]. These devices have been also demonstrated as versatile tools for chemical and biosensing applications [4-6], topic where this proposal is focused and present important advantages compared to previously mentioned interrogation techniques.

Consequently, the utilization of these devices for high-sensitivity demanding biosensing applications is a clear opportunity that could open novel and interesting research lines and applications as well as simplify current analytical methodologies. As a result, on the basis of our previous experience with LMR based sensors to attain very high sensitivities, we propose to study this technology for the fabrication of new biochemical sensing platform and probe its applicability for the detection of microRNA (miRNA) biomarkers associated to rheumatoid arthritis (RA) [7-8].

Therefore, the selection of the adequate miRNA biomarkers and the utilization of rapid and low cost detection techniques for early diagnosis and treatment of rheumatic diseases represents a major challenge in biomedical research owing to the great impact of this kind of pathology in the health system, the society and the economy of the countries.

So, OBUS main objective consist of developing, testing and validating fiber optic sensors based on LMR for the detection of microRNAs associated to RA.

The objectives accomplished in this project are detailed below:

- Selection of a panel of microRNA biomarkers (miRNAs) associated to rheumatic diseases.
- Establish the adequate surface activation and functionalization protocol in order to adhere the adequate biosensing layer with the biomarkers onto the fiber optic LMR-based sensing platform.
- Setup a proof-of-concept of the biosensor and validate its performance in order to iteratively optimize the operation of the device.
- The knowledge generated could be used for detecting single or multiple analytes towards a future multipurpose platform.

2. STATE OF THE ART

The need of medical devices for point of care, early, accurate and real time diagnosis are social challenges to be addressed within the next years. To face them, it is necessary to deeply explore the potential of biomarkers detection as well as the development of simple, cost-effective and real time detection tools and equipment. In particular, miRNA biomarkers have shown a great potential for disease diagnosis but they are still at a very early stage and many technical issues need to be addressed, such as the development of novel technologies that permit a rapid detection at very low concentrations or the evaluation of several biomarkers at the same time. In this sense, qRT-PCR, microarray analysis or massive sequencing techniques have been used but none of them are considered as a valid procedure for routine analysis of these molecules due to the difficulties of the standardization or requiring high microRNA volumes like the case of qRT-PCR, low sensitivity and low specificity like the case of microarray analysis or due to the high associated costs like the case of massive sequencing. In particular, current miRNA analysis techniques offering the scalability, speed, and resolution necessary for evaluating targeted miRNAs of interest employ equipment valued in more than 50.000€. Consequently, a novel detection platform is required to tackle with current challenges and obtain a low cost and rapid detection platform for miRNA biomarkers detection.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

Rheumatoid arthritis (RA) is a polygenic disease characterized by autoimmunity and systemic inflammation with progressive impairment of joints that results in lifelong disability and increased mortality. Early diagnosis and therapeutic intervention or treatment can prevent severe disease manifestations in patients suffering from RA. The use of appropriate predictive biomarkers such us miRNAs, which are modified in the plasma or serum, are considered to be the most promising non-invasive biomarkers for the detection of RA, and may improve the efficiency of RA therapy [9].

Here, the utilization of LMR technology is presented as the alternative of previous technologies. The new configuration of LMR-based devices enable to obtain sensitivities that exceed the list of commercial devices based on different technologies, among which can be found devices from Biacore, Reichert or Fortebio. This technology, presented by the first time by the group of the project coordinator is based on the coupling of modes near the cut-off region and it presents important advantages compared to previously mentioned interrogation techniques as it is enumerated below [10]:

- Label-free: does not require the utilization of fluorescent or colorimetric additional biomarkers.
- Wavelength based detection: robust and immune to optical power fluctuations
- Non polarization dependent: can be used with not polarized light excitation (cheaper)
- Does not require the utilization of a prism: compact fiber optic design
- Simple chemistry: Enable the utilization of metal oxides and polymers for the generation of the resonances, which broadens the possibilities of surface functionalization.
- High sensitivity: 10^{-9} refractive index units[11].

All in all, the multidisciplinary nature of the Project will enable to take advantage of the joint knowledge of optical detection and biosensing application in order to explore the design and development of versatile, compact, rapid and low cost interrogation tools capable of identifying miRNA biomarkers associated to rheumatic diseases. An interrogation tool that consists of the combination of a novel and high sensitive detection technique (LMRs) with the selection of the adequate biomarker panel.

4. PROJECT RESULTS

This section will describe the results obtained in the project until August 31st and comprise: 1) the biomarker panel selection, 2) the fiber optic biofunctionalization steps and 3) the miRNA (the biomarker, miR-223) detection as it is detailed in the following subsections.

4.1. Biomarker Panel

A panel of miRNA biomarkers associated to RA, including their specific matures sequences and chemical properties (see Tab. 1), has been obtained throughout a deep search in literature in order to shown the relevance of miRNAs in RA diagnosis and monitoring [7-9].

Tab. 1. Simplified miRNA biomarker panel associated to RA.

miRNA	Sequence
miR-21	UAGCUUAUCAGACUGAUGUUGA
let-7a	UGAGGUAGUAGGUUGUAUAGUU
miR-16 [12]	UAGCAGCACGUAAAUAUUGGCG
miR-223 [12]	UGU CAG UUU GUC AAA UAC CCC A

4.2. Biofunctionalization steps

A D-shaped SMF fiber sputtered with a SnO_2 thin-film is used as substrate in the biofunctionalization process as it is detailed in [4-6]. All the binding interactions were performed in situ and were continuously monitored. The

measuring setup (see Fig. 1) consisted of a broadband multi-SLED light source (FJORD-X2-1330-1550 purchased from Pyroistech S.L.), an in-line polarizer, and a polarization controller (purchased from Phoenix Photonics Ltd.), which enable selecting TE- or TM-polarized states of light. An optical spectrum analyzer (MS9740A, purchased from Anritsu), is connected to the output to monitor the biosensor response in the 1200-1700 nm range.

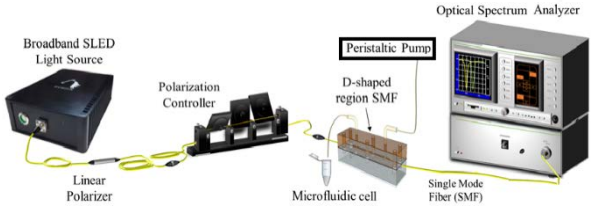

Fig. 1. Schematic representation of the measuring setup

In the first place, the SnO_2 coated D-shaped region of the SMF fiber is immersed in a 0.04% w/v solution of Eudragit® in 99% pure alcohol for 1 min and then it is left drying in air for about 15 min until the solvent has completely evaporated.

After this step, the D-shaped region is placed inside the thermos-stabilized microfluidic cell (see Fig. 1). A peristaltic pump allows to control the flow following the steps detailed in Tab. 2.

Tab. 2. Biofunctionalization Steps.

No.	Matrix	Time (min)	T[a] (°C)
1	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS) solution (2 mM and 5 mM in ultrapure water, 18.1 MΩ/cm)	30	25
2	Streptavidin (STV, 0.03% w/v) in PBS	60	25
3	PBS buffer	15	25
4	PBS buffer	15	32
5	5'-Biotin- TGG GGT ATT TGA CAA ACT GAC A-3' in PBS	15	32
6	PBS buffer	15	32

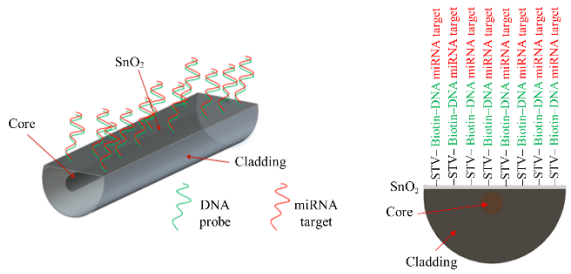

Fig. 2. Schematic representation of the fiber-optic biofunctionalization and sensing principle of the biosensor

Step 4 is done because, ideally, the working temperature must be higher than the melting temperature of both the DNA probe (designed as a complementary DNA sequence of miRNA target) and the miRNA (30.9ºC and 23.1ºC respectively, in this case) and lower than the melting temperature of the hybridization, that is, the binding of the DNA probe and the miRNA (70.9ºC in this case). A schematic representation of the structure obtained after the biofunctionalization steps detailed in Tab. 2 is shown in Fig. 2.

4.3. Detection Protocol

Finally, it must be studied if the biosensor detects the miRNA target, that is miR-223 in this case. Converted DNA sequence of miR-223: 5'- TGT CAG TTT GTC AAA TAC CCC A-3'is used for simplicity in the assays but miRNA could be obtained by simply replacing thymine (T) by uracil (U) (Tab.1). Assay protocol was performed at 32 °C following the steps in Tab. 3.

Tab. 3. Detection Steps.

No.	Matrix	Time (min)
1	Bovine serum albumin (BSA, 0.04% w/v)	10
2	Target miR-223 (0.02 µM)	15
3	PBS buffer	10
4	Target miR-223 (0.02 µM)	15
5	PBS buffer	10

In this case, BSA is employed to avoid non-specific binding of Targert-miRNA. As it is shown in Fig. 3, a 0.02 µM concentration of target miR-223 does not produce an appreciable LMR wavelength shift, whereas a 2 µM concentration of the target causes a shift of 3.05 nm.

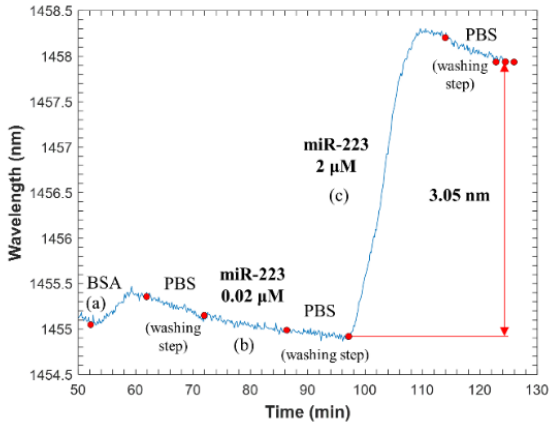

Fig. 3. Sensogram corresponding to the miR-223 detection

At this stage it is clear that the limit of detection of this biosensor, with the current configuration, is between 0.02 and 2 µM, which is within the clinical detection range. However, further research must be carried out in order to precisely establish this value and whether it can be improved or not as well as the fabrication of a multiple biomarker detection platform.

5. FUTURE PROJECT VISION

OBUS aims at developing a new multiple biomarker photonic platform for early diagnosis of diseases, capable of providing a rapid and accurate point of care assessment. using small volumes of biofluids (blood or urine) with femtomolar (fM) limit of detection (LOD).

5.1. Technology Scaling

To this purpose, a new breakthrough technology based on LMRs) [1-6] is proposed. Here, further research is required to improve the detection limit, the optimal working temperature for the biofunctionalization and detection processes as well as an in depth study of the target and receptor affinity and cross-sensitivity with other molecules. Concerning the platform, it is also necessary to improve the integration of multiple photonic detection channels within the same device by means of micro and nanofabrication techniques.

5.2. Project Synergies and Outreach

OBUS is a multidisciplinary project that comprise physical, biological, chemical, medical and photonic sciences. The IP has strong contacts with important players from academia and SMEs in these areas, which will be essential for the success of the project goals.
In addition, all the players have a strong tradition of both disseminating and exploiting the scientific results in the form of papers, patents and spin-off companies.

5.3. Technology application and demo cases

Proposed technology will be demonstrated in the precision diagnostics of RA using a multiple biomarker panel with 4 miRNA biomarkers [7-9]. This analysis will prove the validity of this platform and will open the path towards the detection of other illnesses.

A disruptive impact on society is expected through the development of this project bringing a better quality of life for citizens. It is also expected to be a helpful tool for clinicians reducing the costs of public health. On the economic context, it is expected the new technology provided by OBUS will not only generate knowledge in academia but also be transferred to the industrial sector.

5.4. Technology commercialization

SMEs in the medical instrumentation area have already shown particular interest in the results obtained from the project in order to provide a novel type of medical equipment easy to handle, small in size, portable and of general purpose.

5.5. Envisioned risks

OBUS is supported by a deep knowledge on the fundamentals of different sciences provided by the partners. A consortium of experts specialized in different areas will be created with the purpose to mitigate the high risks associated to the different project stages, such as the biofunctionalization protocols, the detection process, the photonic integration or the medical regulations.

5.6. Liaison with Student Teams and Socio-Economic Study

OBUS project is also interested in collaborating with Student Teams for providing ideas and prototypes inspired by the project technology as well as addressing Societal Challenges. In this sense, a project member will be coordinating all these actions at all the stages during the project duration in the form of internships and PhD programs as well as providing information of the project in the form of videos, interviews and press releases.

6. ACKNOWLEDGEMENT

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