"Single molecule bio-electronic smart system array for clinical testing"

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PUBLISHABLE EXECUTIVE ABSTRACT

The SiMBiT project aims to develop a bio-electronic smart system that can perform single-molecule **detection of both proteins and DNA bio-markers**. Specifically, the SiMBiT activities will develop the lab-based device into a **cost-effective** portable multiplexing array prototype with extremely **fast time-to-results**.

The first year of the project at the consortium level was dedicated to the set-up of the product's specifications, common procedures and protocols. Each WP and partners worked on the design and the characterization of the semiconductor, the EG-TFT as well as the design of the final prototype. During this first year, the set-up of the collection of the biologic samples and miRNA identification was planned.

TECHNICAL REPORT – WP OBJECTIVES FROM M1 TO M12

WP1 OBJECTIVES FROM M1 TO M12

The Work Package 1 is devoted to the Technical requirements preparation of the Simbit device including electronic, mechanical and software parts (Task1.1). Further, another task (Task 1.2) is the preparation of standard procedures and regulations and finally quality control assessment for the project activities (Task 1.3).

WP2 OBJECTIVES FROM M1 TO M12

The work package 2 is dedicated to the fabrication, characterization and optimization of the single sensing device. During the first year of project, the efforts were focused on the optimization of the organic semiconductor channel material and the gate design and performances, hence on the two pillars at the basis of the SiMBiT prototype. The main objective for the activities on the channel material was the identification of the most situated organic semiconductor, compatible with large area printing technologies, able to stably operate in water. As far as the gate is concerned, the activity carried out aims at the demonstration and validation of a 3D printed gate structure suitable for the development of the final gate cover plate that will fit the standard ELISA plate geometries.

WP3 OBJECTIVES FROM M1 TO M12

In M1 – M12 the WP3 focused on the characterization of electrolyte-gated thin-film transistors (EG-TFTs) including electrical and optical analysis by varying the geometrical, physical and chemical characteristics of the semiconductor, electrolyte and gate. The EG-TFTs specifications accounting for the single device architecture as well as the matrix implementation are defined. The device physics has been investigated and the main figures of merit of EG-TFTs have been identified. These results will support the optimization of the single EG-TFT bio-sensor as well as the design of the sensor matrix and will be used for the validation of the models currently under development.

WP4 OBJECTIVES FROM M1 TO M12

From M1 to M12 the main activities of WP4 were focused on the design, characterization, optimization and validation of a single EG-TFT sensor based on a printed semiconductor fabricated with large-area

processes compatible with the realization of a planar 8 x 12 array plastic foil. The electrical characterization of single EG-TFTs was carried out in order to asses compliance to the performance and specifications defined in D1.1. There results supported the design of an integrated array with 4 x 4 EG-TFTs comprising the output pins for the integration with the addressing electronics from WP5. All the activities related to the fabrication of the 4 x 4 integrated array started on M10.

WP5 OBJECTIVES FROM M1 TO M12

The objective for WP5 between M3 and M12 is to complete the system design for the first 4x4 well array SiMBiT prototype. During this activity it is critical to consider the design of the final prototype as well as the complexity and manufacturability. The prototype consists of disposable and non-disposable elements; thus, it is important to consider the cost and keep higher cost items as part of the non-disposable part. The components specified as part of this design include the bio-electronics sensor array, addressing electronics exploiting organic TFTs on foil, a frontend Si chip, a readout PCB, software interface on the PC and the interface between the SiMBiT system and the MASMEC fluidic handling machines.

WP6 OBJECTIVES FROM M1 TO M12

Work Package 6 is dedicated to sample collection and analysis of clinical samples by next generation sequencing (NGS) and ELISA. These state-of-the-art diagnostic methods will be compared to results obtained by the SiMBiT device. A further objective is the screening for possible additional biomarkers in pancreatic cyst fluids, which could be analyzed by SiMBiT in the future. To fulfill the objectives, this first year has been dedicated to establishing the logistic for patient recruitment and study inclusion, as well as to timely analyze the clinical samples.

WP7 OBJECTIVES FROM M1 TO M12

The Work Package n°7 is dedicated to communication, dissemination and outreach activities. One of SiMBiT communication and dissemination objective is creating awareness, raising interest, fostering engagement and accelerating the market uptake. To do so this first year was dedicated to the creation of a visual identity for the project, develop online and physical supports of communication and dissemination activities and to outreach on partners attending to external events.

TECHNICAL REPORT – WP PROGRESS AND ACHIEVEMENTS FROM M1 TO M12

W1 PROGRESS AND ACHIEVEMENTS DURING M1 TO M12

The WP1 consists of three tasks:

Task 1.1 Defining hardware and software specifications for the SiMBiT prototypes as a clinical point-of-care platform suitable for a fast application of International Organization for Standardization procedures and regulations.
Task 1.2 Defining of Quality Control and Quality Assessment (QC/QA) procedures that will be produced for a medical device certification.
Task 1.3 Monitoring that the activities carried out in the other WPs to comply with the technical specifications and procedures identified in WP1.

In the T1.1 the hardware and software specifications for the SiMBiT prototype have been prepared. All partners have defined the main requirements related to the functionality of the bio-sensor physical architecture for usability inside a point of care platform. This activity has been completed.
In the T1.2 standard procedures for QC/QA have been analized and a check list tool was prepared, the QC check list is produced on the partner by partner basis every three months. The T1.2 activity has been completed.

In the T1.3 the monitoring activity is performed using the QC check list tool designed in the T1.2. Every three months the partner check lists are compiled and controlled. The T1.3 will be completed at M42 when all the check lists will be closed and available for certification activities.

W2 PROGRESS AND ACHIEVEMENTS DURING M1 TO M12

In the first twelve months we developed and submitted two deliverables in due time:

D2.1: Printed EG-TFT comprising the nanostructured organic semiconductor, stably operates in pure water for at least 7 days. The gate is the reference gate. It was monitored the behavior of the poly(3-hexylthiophene-2,5-diyl) (P3HT) when is used as channel material in EG-TFT. The effects of a prolonged interaction between P3HT and water were characterized using surface techniques as X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM), and it was studied how these changes in the semiconductor morphology and composition affects the EG-TFT performances. The P3HT processing parameters were optimize in order to guarantee the fabrication of printed EG-TFT with elevate performances and able to stably operate for a prolonged time. Part of these results have been published (https://www.frontiersin.org/articles/10.3389/fchem.2019.00667/full).

D2.2: Report on design and development of “3D arrays of gates”. In this document we report the design, fabrication process flow and the development of 3D arrays of gates. The activity performed aims at the demonstration and validation of a 3D printed gate structure suitable for the development of the final gate cover plate that will fit the standard ELISA plate geometries. More in detail, first the specifications for the 3D printed gates are provided, then the adopted printing technology and the design flow are presented. The key steps of the design flow are analyzed by designing specific structures, as well as material and electrical analysis. 3D printed gates suitable for the scaling to the final 8x12 gates arrays are demonstrated.

W3 PROGRESS AND ACHIEVEMENTS DURING M1 TO M12

During the period M1 – M12 the evaluation of the figures of merit and device physics of the electrolyte-gated thin-film transistors (EG-TFTs) have been achieved. The impact of the geometrical, physical and chemical characteristics of the semiconductor, electrolyte and gate on the EG-TFTs electrical characteristics and stability have been analyzed both experimentally and theoretically. A quasi-two-dimensional EG-TFT model able to reproduce the electrical characteristics of bio-functionalized EG-TFTs as a function of the ligand concentration has been developed and validated. This model provided insight on the bio-functionalized EG-TFTs and it is currently exploited in order to obtain an analytical formulation. Various tools for the EG-TFTs simulation and optimization are currently under implementation.

W4 PROGRESS AND ACHIEVEMENTS DURING M1 TO M12

IIT optimized and validated a process flow for the fabrication of single Electrolyte-Gated Thin Film Transistors (EG-TFTs) compatible with the realization of the final array. As planned, an array of 4x4 devices was also developed with the same process.

The main steps involved in the process are:

- The realization of gold contacts on plastic flexible substrates;
- The selective electrical insulation of such contacts;
- The deposition of the organic semiconducting polymer.

Gold Contacts. In order to realize the contacts, two strategies have been considered and tested:

i- Gold thermal evaporation through a shadow mask and successive laser ablation of the micrometric channel area
ii- Photolithography

Owing to the better results obtained with photolithography, especially in terms of reproducibility, and to an expected easier implementation at an industrial level, in agreement with partners it was decided to choose and further optimize option “ii”. Contact pads for both single devices and 4x4 arrays were successfully realized with good reproducibility.

Electric Insulating Layer Deposition. The deposition of a dielectric layer is necessary to insulate the gold contacts and to reduce electric current leakage from source/drain contacts towards the gate.

This process has been carried out by inkjet-printing an insulator only in the areas required. Careful optimization of the process parameters allowed to exploit the inkjet printing technique to design the matrix layout in such a way that all contacts are presented only on one side of the array, facilitating the integration with the external reading electronics.

Organic Semiconductor Deposition. The deposition of the semiconducting layer was carried on via inkjet printing as well. Again the optimization of the process parameters and of the substrate cleaning and preparation protocols allowed the realization of devices through a large area and scalable technique.

W5 PROGRESS AND ACHIEVEMENTS DURING M1 TO M12

During the period between M3 and M12 the objective to complete the system design for the first 4x4 well array SiMBiT prototype has been achieved. A high level system design overview has been defined and discussed in a deliverable report. More detailed designs of each component has been realised, including the bio-electronic sensor array, addressing electronics exploiting organic TFTs on foil, the frontend Si chip and the non-disposable readout PCB. A critical aspect of the first SiMBiT prototype design is the mechanical implementation, more specifically, the interfaces between each component. This has been discussed between project partners and defined, with any associated risks and risk mitigation options identified. The process flow in which the first SiMBiT prototype will be created has also been determined.

W6 PROGRESS AND ACHIEVEMENTS DURING M1 TO M12

First, we established functional logistics for clinical sample collection with two clinical collaborators. All collaborators have been informed about the criteria of study inclusion and ethical requirements for

each patient. Aliquots from incoming samples have been stored for later testing with SiMBiT. Simultaneously, all clinical samples have been analyzed by Next Generation Sequencing (NGS).

The screening for additional biomarkers has been focused on expression of the protein MUC1 and of several miRNAs. If sample volumes allowed, expression of MUC1 has been determined by ELISA and expression of miR-21-5p and miR-221-3p has been determined by qPCR. To screen for more potential miRNA candidates, miRNA-sequencing on pancreatic cystic tumor tissue has been performed.

For this first year, we can report a clinical collective of 37 patients. All samples have been analyzed by NGS, in 18 samples ELISA was performed. As a result of the biomarker screening, we can outline MUC1, miR-21 & miR-221 as promising biomarker candidates, as they are upregulated in PDAC and mucinous lesions.

W7 PROGRESS AND ACHIEVEMENTS DURING M1 TO M12

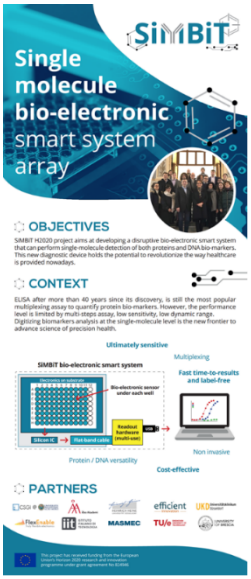
A set of public communication tools was developed during the first months of the project and updated throughout the project duration. SiMBiT partners attended to several workshops, international conferences and other relevant events presented good opportunities to disseminate and discuss

The due deliverables for this year were submitted online in due time:

D7.1 : Report on the public Website and Social Media profile Setup. The first step of dissemination activity was the creation of a logo, a public website and social media profile, so everyone could access to project presentation, objectives and consortium description. This website/profile will be used all along project duration to support communication via uploaded public deliverables, links to possible scientific publications and announcement of consortium activities.

Early on in the project a SIMBIT public website (simbit-h2020.eu) and social media profile on LinkedIn (https://www.linkedin.com/company/36020918) has been developed and has been continuously updated throughout the entire year. For the first year the News & Events section has been updated five times with information about performed SIMBIT-related publications, upcoming relevant Events, meetings and Workshops. We also following closely the activity on the website.

D7.2: SiMBiT leaflet and poster. SiMBiT partners is taking part of dissemination activities as trade exhibitions, conferences etc. To support communication, leaflets and posters will be distributed or displayed during relevant events (see below).



On the dissemination level we can report for this first year of the SiMBiT project about 200 users of our website, several publications on news & events as well as on LinkedIn SiMBiT page, 16 participations to international and European congresses and conferences and 5 participations to workshops.

CONCLUSION

The first year of SiMBiT project has been successful, all deliverables have been submitted in time and the work follows the planning. The management and dissemination of the project has been set-up as well as the common procedures and characterization of the final product. The physical characterization of the EG-TFT has been pursued and the simulation and optimization of the system are under implementation.

APPENDIXES