# SMILE - A Saw-Mip Integrated device for oraL cancer Early detection

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***ABSTRACT***

Head and neck cancers represent the 6th most common type of cancer in Europe and if not diagnosed at the early stages, when curable lesions are asymptomatic, invasive surgery is needed. To improve the quality of diagnostic early screening and increase compliance of patients, SMILE project focused on the detection of biomarkers from *saliva* by -innovative transducer (*Surface Acoustic Waves*), -smart materials mimicking biological capture probes (*Molecularly Imprinted Polymers*) and -label-free assay. From the core work, collateral activities arose: *low-cost microfluidics* to detect oral cancer circulating cells and last but not least, SMILE project started dealing with COVID-19 related activities.

*Keywords: Oral Cancer early diagnostics; Personalized medicine; Key Enabling Technologies.*

## 1. INTRODUCTION

The main objective of SMILE project has been the development of a low-cost sensing device for Oral Squamous Cell Carcinoma (OSCC) early screening, with features of high sensitivity, portability and ease of use, with the aim to reach a large audience. OSCC is usually diagnosed at an advanced stage, where highly invasive surgery and chemotherapy are required, heavily compromising life quality and survival [1, 2]. Early detection of OSCC is the only way to limit consequences of disease and the main challenge in prevention is large-scale screening. To reach this goal, the needing for non-invasive assay is compulsory and the possibility to perform tests on saliva is an attractive strategy to make this test suitable for all, as well as the ambitious objective of SMILE project.

To reach these goals, SMILE project put in place a combination of innovative tools and methods for transduction technology, capture probes and microfluidics in a highly interdisciplinary project (Fig.1). This has been possible thanks to the contamination between expertise in various background carried out by a young and well-integrated working team. Breakthrough technologies of the platform include the optimization of artificial antibodies, sensitive transducer methods integration and highly customizable microfluidics. The special combination of these features in SMILE project have been realized thanks to the partnership between CNR NANOTEC Institute, devoted to nanotechnologies applied from smart materials to precision medicine and STMicroelectronics, a company dealing with the development of smart and prospective technologies for medicine and life sciences. Activities carried out during the project have contributed to an advancement with respect to the state of the art in the field of diagnostics, fabrication methods and sample handling.

In particular, we achieved:

- the modelling and realization of the Surface Acoustic Waves (SAW) transducer;
- the integration of SAW with electrosynthesized Molecularly Imprinted Polymers (MIP) for Interleukin-10 (IL-10) and C-Reactive Protein (CRP) as sensing element and their calibration down to 1 ng/ml, corresponding to picomolar concentrations, useful for cancer diagnosis [3];
- the analysis of spiked samples of artificial saliva to test and validate the assay;
- highly customized plastic microfluidics for circulating tumour cells detection.

The compelling situation of COVID-19 interrupted the normal course of the project but from difficulties often new opportunities rise up, and we took the chance to partially modify the developed tool and skills to give our contribution to the scientific community in these hard times for public health.

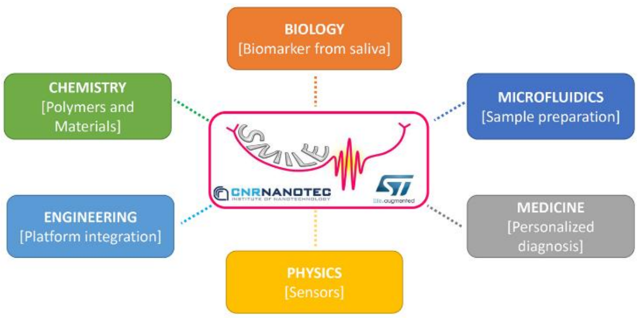


**Fig. 1.** Interdisciplinary character of SMILE Project

## 2. STATE OF THE ART

SAW based devices, a well-established technology in the field of transmissions and electronics, are gaining interest also as tool for sensing platforms, due to their very high sensitivity to what happens at the SAW propagating line[4]. This indeed can strongly affect shape and phase of the SAW which can be detected by the receiver. Thanks to this working principle and the possibility to functionalize the propagation surface with biological probes able to capture analytes (i.e. antigen/antibody), biorecognition phenomena can be deeply investigated[5]. MIP are a class of smart materials able to act as artificial antibodies. They polymerize in presence of a template (analyte); once it is removed, its molecules leave cavities in the bulk able to rebind the analyte if it comes again in contact with the polymer, with high degree of selectivity. Moreover, the possibility to directly polymerize MIP at the surface of electrodes through electrochemical methods (cyclic voltammetry)[6], makes this class of polymers particularly suitable to their integration in miniaturized systems.

IL-10 is gaining a strong interest as a diagnostic and prognostic marker for the early detection of OSCC. Its presence in biological fluids, in particular in saliva, is associated with the disease, while very low in healthy controls. IL-10 is an immune-modulator molecule[7] used as a marker for inflammation and cell proliferation. CRP has been selected as positive control molecule, less specific for cancer, but detectable in the case of inflammation[3].

Finally, microfluidic technologies and use of plastic substrates in combination with rapid prototyping methods is appealing alternative in the realization of polymeric lab-on-chips[8]. The combination of femtosecond laser based micromanufacturing and micromilling technologies paves the way to the realization of highly customizable devices with high level of flexibility necessary to push forward the realization of tools for handling unconventional body fluids like saliva.

## 3. BREAKTHROUGH CHARACTER OF THE PROJECT

SMILE project proposes a platform able to integrate some disruptive technologies and strategies for the early screening of oral cancer, encompassing innovation in many fields: *(i)* innovative transducer method with features of high sensitivity; *(ii)* smart materials ensuring high selectivity as sensing elements in the shape of artificial antibodies; *(iii)* IL-10 selected for its high specificity and affordability as prognostic and diagnostic biomarker. Current detection methods to identify and quantify biomarkers of pathologies in biological fluid are based on standard biochemical assays like ELISA which are time-consuming, requires heavy benchtop instruments and specialized and highly trained personnel. Our SMILE platform aims to accomplish the "*sample-in/answer-out*" technology by realizing a compact and easy-to-use platform which can be provided to healthcare professionals in order to reach a large cohort of screened people. The developed platform integrates high sensitivity SAW transducers (able to reach detection limits under the nanogram concentrations through immunosensing[9]), with MIP technologies, that have the potential to strongly push forward the sensing capabilities, beyond the common detection limits of antibody-based assays and taking also advantage on their synthetic nature, which allows high stability and no-need for cold storage. As a matter of fact, the imprinting and subsequent rebinding of a polymer is able to distinguish among enantiomeric molecules[10] and to selectively identify small analytes. In such a scenario, the detection of a low molecular weight protein like IL-10, a promising biomarker for OSSC, combined with the high capability of SAW/MIP technology, strongly go beyond the state of the art of biosensors and preventive medicine. To the best of our knowledge, nothing incorporating such innovative items is currently available on the market, and only few research papers have been published in the related literature.

Recently, during COVID-19 emergency, FDA released the approval for home collection of saliva samples[11], in order to avoid crowdy group waiting for nasopharyngeal swab tests, decreasing also the risks of infection for medical personnel. So, the need for a tool able to store, stabilize and prepare saliva sample for analysis is compulsory, and SMILE project, based on the expertise developed on artificial saliva handling, is working also in that direction.

## 4. PROJECT RESULTS

SMILE project explored several aspects of technology, spanning from simulation of the entire platform through Finite Element Methods (FEM), to tests with artificial samples, to the use of microfluidics using oral cancer cells. Each of these has been deeply investigated and results will be presented below.

**FEM modelling of SMILE platform**

The behaviour of the devices has been studied by implementing a mathematical model in *Matlab*, allowing to test design parameters and monitor relative figures of merit. A device FEM model has been performed in *COMSOL*, in order to obtain compliance with experimental data.

Several settings and boundary conditions have been investigated to obtain models whose behaviour is close as possible to the experimental one. Frequency domain simulation result relative to the single delay line structure, with the presence of the electrode for the MIP

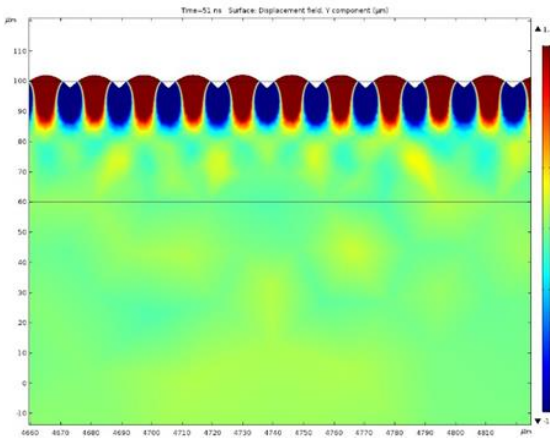

**Fig. 2.** Vertical surface displacement at transmitter interdigitated electrodes

electrosynthesis has been compared to the experimental measurement of the homologous device. The COMSOL model frequency response is obtained applying Matlab FFT routine to the time domain signal. The surface deformation can be observed in Fig.2 and the resulting spectrum has been found smooth and regular, according to measured one. The obtained frequency response peak is centered at the same frequency value as the measured one, with a difference of 61 kHz. The peak amplitude of the spectrum presented a reduction by the 35.5% with respect to the experimental data (data not shown)[12]. probably due to the mechanical damping which has been estimated.

**Realization of the SAW transducer**

SAW-based transducers were realized on Lithium Niobate ($LiNbO_3$) substrates by means of a lithographic process performed through a Direct Laser Writing system (Heidelberg Instruments) processing CAD/Clewin® designs, providing couples of interdigited electrodes to arrange delay line with microfabricated electrodes for Cyclic Voltammetry (CV) in between.

**MIP optimization and integration**

Electrosynthetized MIPs were obtained on the surface of central microelectrodes through a CV process which allowed to polymerize imprinted (with IL-10 and CRP embedded into a film of ortophenilendiammine - OPD) and non-imprinted polymers directly in the middle of the delay lines. The process is highly reproducible and allows the perfect confinement of the polymer on the surface of the electrode.

**Testing of the SMILE platform**

The obtained devices with imprinted (MIP) and non-imprinted polymer (NIP) were washed to reveal active sites and incubated with the related protein solution, achieving the detection of less than 1 ng/ml for both biomarkers, corresponding to concentrations of 8pM for CRP and 56pM for IL-10[13]. Protein solutions were prepared both in PBS and artificial saliva and a buffer protocol in order to optimize unspecific adsorption derived from saliva components has been investigated. The microfluidic module impact on signal is also under analysis, with the aim to integrate a sample handling module into a compact platform able to provide a diagnostic result based on the detection of biomarkers (the peak of the curve decrease proportionally to increasing concentration of analyte) (Fig.3).

**Microfluidic tools for oral cancer detection**

Microfluidic modules were produced and adapted to the multiplexing platform planned in SMILE project, but in parallel, technologies arising in the project frame and optimized taking advantage of methods like micromilling manufacturing, were applied to another experimental setup. In this case manufacturing technology included also femtosecond laser technique, able to produce microchannels with high precision and transparency. The combination of the two manufacturing methods allowed us, together with an optimized thermal and solvent-assisted bonding, to obtain a tool prone to the biological functionalization to capture oral cancer- derived circulating tumour cells (CTC) from an heterogeneous sample[14].

**COVID-19 commitment of SMILE Project**

After the release of the American Food Drug Administration which recently authorized the use of home-collected saliva to detect SARS Cov-2, patients are allowed to self-collect saliva samples for analysis in order to improve the accessibility to COVID-19 testing and decrease the risks of infection for medical personnel. In the frame of SMILE project, STMicroelectronics and CNR NANOTEC, already focused on the detection of biomarkers from saliva samples and dealing with artificial saliva mimicking real samples, are investigating the possibility to develop a plastic disposable tool able to stabilize, preserve and prepare saliva samples also usable for COVID-19.

## 5. FUTURE PROJECT VISION

SMILE vision has been focused on the realization of a platform for rapid detection of oral cancer biomarkers with the aim to reach a large audience. What can be challenging now toward a self-contained tool is *on-chip* sample preparation, enrichment and purification, which has emerged as a very important task in handling non-conventional samples like saliva, as also emerged from recent pandemic conditions. To reach these challenging aims toward optimizing a *sample-to-answer* system prototype, we plan to manage a 3-years project, involving 5-7 partners (two from Attract projects).

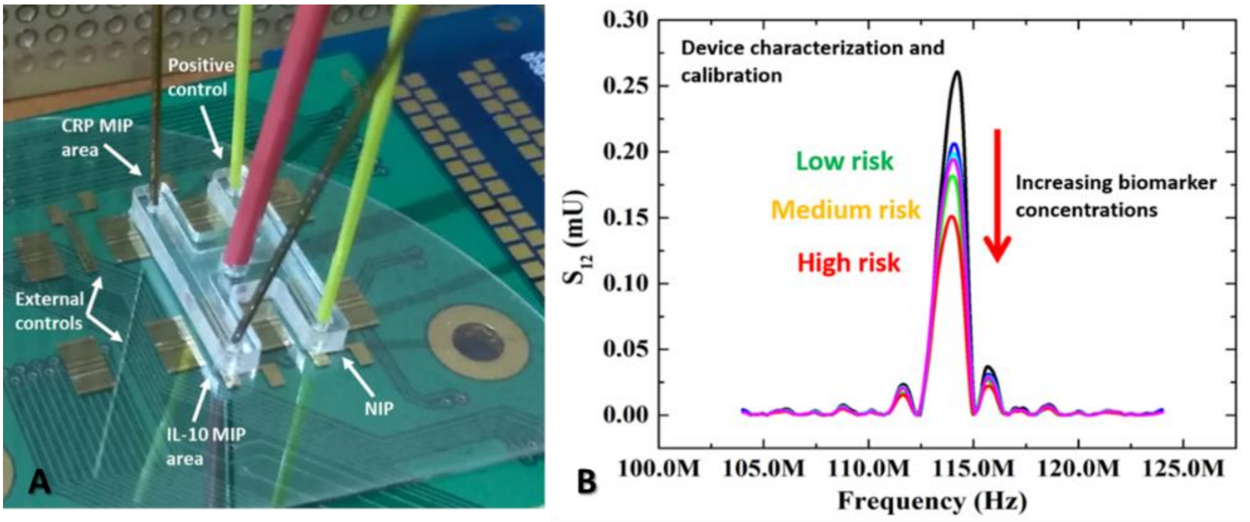


**Fig. 3.** A: SMILE platform with highlighted SAW sensing areas and microfluidic module made by micromilling technology able to deliver saliva solution for analysis directly on the surface of MIP-functionalized electrodes. B: Working principle of sensor with increasing biomarkers concentration associated to a higher risk for oral cancer

### 5.1. Technology Scaling

Scaling up of project technology during Phase2 is based on STMicroelectronics that plays a role in industrial exploitation. With the aim to move from a TRL3-4 gained in Phase1 to a TRL5-7 during Phase2, we will fix some milestones which are core achievements of our project and implement high throughput suitability, low costs and low environmental impact. Fundamental step to demonstrate a real applicability is to distribute devices among scientific partners and/or selected final users. These will represent the *beta testers* that will help to improve the devices, tuning their features toward *key enabling technologies*.

### 5.2. Project Synergies and Outreach

Our vision is exploiting our experience acknowledged in Phase1 and integrate MIP capture probes into microfluidic customized device, able to handle biological samples, add and mix reagents, capture and detect target biomarkers. Combining skills and expertise of the consortium, each of these new tasks will be released as soon as it will be implemented as an additional function of a modular platform.

In particular, we will

- modify chemical properties of MIPs making them pH/temperature responsive: once biomarkers will be captured from a certain volume of sample, it will be sufficient changing physical conditions and MIP will release the molecules for analysis. The handle of liquid samples and reagents will be managed by silicon MEMS technology. A MIP-based diagnostics company get interest for our approach and it will be included it in the consortium.
- modify and adapt architecture of the device through micromanufacturing expertise already involved in the project.
- integrate rapid and miniaturized optical detection methods thanks to collaboration with two Attract projects in the field of photonics and a European research group, leader in photoacoustic spectroscopy for lab-on-chip.
- realize a risk-assessment analysis and patients stratification by means of a new partner in the field of machine learning and Artificial Intelligence

### 5.3. Technology application and demonstration cases

Phase1 was designed to realize a tool allowing clinicians reach a large audience and fast and safe management of patients. Thanks to the highly customizable design, the system can be easily transferred in agriculture, food, veterinary areas and COVID testing.
This will include plastic microfluidics realized by innovative smart technologies for the on-chip management of saliva samples to easily detect molecules with specific disposable cartridges. The plan is to include pre-loaded reagents *on-chip*, to automatically process the sample. This tool will be in principle useful for other unconventional samples with the possibility of having wide application.

### 5.4. Technology commercialization

Developed devices and sample preparation modules we want to improve, identify a "*general purpose technology*". Market suitability may vary depending on the rules that each sector requires: an application to agriculture needs less certifications than a veterinary one, which need less clinical tests than a healthcare IVD tool. Our focus remains early diagnosis of diseases with an extra-scope with Covid-19. Know-how generated during the project will be protected and patented by SMILE consortium. The presence of at least two companies, key players in diagnostics market, will pave the way to the technology transfer of SMILE systems.

### 5.5. Envisioned risks

The achievement of SMILE goals is possible by combining multiyear expertise of the consortium partners, which has already skills in all the proposed technologies and the partnership is well consolidated as demonstrated by some previous publications dealing with SAW sensing devices[5], materials[16] and lab-on-chip[17]. This would allow the identification as "low-risk activity" also for Phase2 management. In any case, to minimize activities-related risks, we plan to start from tackling simpler applications, with single module release (channel network, mixer, detection, waste), which may be more attractive from a stakeholder's point of view as a low-risk and less complex process to pass from laboratory to everyday practice. Successively and in parallel, the suitability of the platform to multiple biological fluids and/or specific biomarkers will be investigated with the possibility to shift to high throughput production.

### 5.6. Liaison with Student Teams and Socio-Economic Study

SMILE project paid attention to young scientists and students at the beginning of their research career. SMILE team included three students (from different nationalities) that have significantly contributed to project activities: a MS from Polytechnic of Turin, a PhD student of University of Salento (SMILE fellowship), and one from University of Bari. The course in "Nanobiotechnologies and biosensors" at University of Bari, hold SMILE technologies contents. A workshop "EDGE-Tech-Emerging and Disruptive next-GEneration Technologies" was planned involving representatives of ATTRACT ecosystem and international high-level speakers. Maintaining a fruitful communication is vital for the project. To this aim social network were active throughout the project reaching a high number of contacts and visualizations, with the dual intent to have a high impact on early prevention of diseases, and to reach the youngest bracket of the population to be inspired to entrepreneurship-committed research. With this vision, collaboration with SMEs or potential stakeholders that can act as an Advisory Board would be the gold standard.

## 6. ACKNOWLEDGEMENT

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