PROJECT RaFrBio

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EXTERNAL INSTITUTIONS
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Executive summary

Mission Statement

According to recent studies led by the World Health Organization, the number of patients affected by diabetes has been constantly growing at alarming rates since the past few decades, mainly due to population growth and increase in population average age. In order for medicine not to lag behind such an ever-expanding phenomenon, new easy-to-use, low-maintenance glucose meters should be devised. As there is also much interest in abandoning the traditional blood-measuring approach due to its invasiveness, novel sensing techniques are required as well. Radio Frequency (RF) sensors represent an innovative solution in this sense, since their high sensitivity allows them to painlessly measure glycemia from sweat or ocular fluid, where glucose concentrations are typically lower, rather than from blood. Such high sensitivity is achieved by means of substrate functionalization of the antennas, that is a chemical modification process favoring glucose molecules binding to sensors surface. RaFrBio project tackles this issue on a set of already existing RF sensors (patch antennas), employing an avant-garde technology, i.e. graphene-enhanced interaction.

Sensor Functionalization

The biggest challenge faced during the project has been the design of a functionalization procedure which could provide the sensor with both selectivity to glucose only and good sensitivity in terms of resonance frequency shift versus glucose concentration. The starting point was a compact patch antenna of droplet-compatible dimensions, built by Prof. Savi at Politecnico di Torino. Due to its dimensions, such antenna has a resonance frequency in the range of few GHz. A tailor-made functionalization routine has been developed, involving the deposition of a layer of graphene as coupler for the later deposited glucose binding protein (GBP) or glucose oxidase (GOx), molecules that are highly reactive to glucose. A functionalized sample antenna has been sent to the "Istituto Italiano di Tecnologia" (IIT) facilities for validation, with very promising results. In order to allow for a simultaneous characterization of different deposition techniques, a custom multi-channel measurement station has been prototyped: it consists of a portable, user-friendly instrumentation, hosting up to four frequency-measuring electronic boards entirely designed by the team itself. All these electronic devices are highly modular, meaning that they can be deployed for the measurement of other molecules than just glucose with very little effort. Such electronic system has shown to be perfectly compliant with the sensitivity data extracted from the above antenna.

From Lab to Market

To have a firmer grasp on the possible position of the solution within the wide market of biosensors, an exhaustive study regarding state-of-the-art technologies has been led. This, together with a visit at MEDTEC 2018 in Stuttgart, one of the largest expositions of innovative medical devices in Europe, highlighted the lack of a RF approach among the existing sensing methods. As a result of the team's increased awareness of RaFrBio innovation potential, a detailed study about the solution patentability was conducted. Consulting a set of online patent databases, the sensor emerged to be the best candidate, pushing the team into contacting the Technology Transfer office of Politecnico di Torino. A custom-made survey concerning glucose meters was handed to both users and experts of the field in order to select successful drivers for the future product development. The collected results pointed out the demand for features like continuous monitoring, user-friendliness and reduced maintenance. Such features will be surely taken into account for the future development of a market-ready product.

Keywords

Diabetes Radiofrequency Graphene Glucose Sensor

Project description written by the Principal Academic Tutor

The development of RF biosensors for biomolecular detection is a very recent trend and is still in the early stages of definition.

The introduction of carbon-based nanomaterials thin film seems promising not only for the detection of biomolecules but also for the realization of passive biosensors for diagnosis of various cancers (breast, prostate …) as well as routine clinical analysis (detection of glucose levels in the serum or drug detection and monitoring).

Clearly, the realization of RF sensors with high sensitivity and low detection limits requires multidisciplinary research.

The goal of this project is to realize a small size, lightweight, biocompatible and cheap radio-frequency sensor for bio-sensing applications. The performances of the sensor will be improved with the introduction of carbon-based thin films.

In the first part of the project, a specific application and the most promising configuration of the sensor will be chosen. The sensor will then be designed and realized using standard PCB etching techniques. The carbon-based nanomaterials thin film will be deposited on the sensor by means of screen-printed electronics manufacturing technology. In the final part of the project, the sensor prototype will be tested for the chosen bio sensing application.

In this multidisciplinary project students will have the unique opportunity to be involved in a new research field and to give a contribution for future technological and medical advancements.

For the detection of various biomolecules, many invasive techniques such as electro-impedance spectroscopy, enzyme oxidation, time domain reflectometer, and surface plasma resonance exist.

Recently, the use of radio-frequency (RF) biosensors based on passive and/or active devices and circuits has been investigated. The performance of these biosensors can be enhanced by the introduction of nanomaterials.

In this project, we focus on passive RF biosensors (meander lines, interdigital capacitors, or microstrip patch antennas) designed for detection of glucose levels in the serum or other applications. The performances of the printed sensors will be enhanced by the addition of nanomaterials (e.g., carbon nanotubes, graphene oxide, magnetic and gold nanoparticles, etc.) on the surface by a screen-printing technique. In this technique, a mesh is used to transfer ink onto a substrate except in the areas made impermeable to the ink by a blocking stencil. The surface will be properly functionalized.

It has several advantages:

- it works on different substrates (plastic, glass, flexible substrates…).
- it is a low cost and it is easy replicable
- various carbon-based filler can be added to the ink.


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Team description by skill

- Nicolò Bonacina: M.Sc Electronics Engineering, worked on the full-stack development of the hardware electronics.

- Davide Carminati: M.Sc Materials and Nanotechnologies, worked on the materials and chemistry related with the sensor and its functionalization.

- Matteo Collura: M.Sc Nanotechnologies for ICTs, worked on the patentability analysis and the front-end software development.

- Stefano Gabetti: M.Sc Biomedical Engineering, worked on the state of the art research, market analysis and back-end software development.

- Massimo Giordano: M.Sc Nanotechnologies for ICTs, worked on the physics of the sensor and the front-end software development.

- Andrea Sottocornola: M.Sc Electronics Engineering, worked on the full-stack development of the hardware electronics.

- Lorenzo Vergari: M.Sc Nuclear Engineering, worked on the market analysis, the related survey and back-end software development.

Goal

With the number of patients affected by diabetes rising at sustained rhythms, the development of new easy-to-use, low-maintenance glucose meters has become an important topic of research in the biomedical sector. In the light of such a demand for innovation, the aim of this project is to introduce a novel technology for the sensing of biomolecules, with a particular focus on glucose.

Team “RaFrBio” (namely, Radio Frequency Biosensor) decided to approach the world of life sciences to give its contribution in research, with the aim of building a prototype of a (possible) medical instrument.

The scientific research plays an important role in the whole project; however, this is not the only. The reason why most technologies today are successful is not strictly related with the engineering aspects, but rather on the trade-off between progresses in research and marketing campaign, that comes along with product and market analysis.

This is why the team faced the challenge of bringing a purely scientific research activity towards the market, eventually building an instrument capable of measuring the glucose concentration in a fluid.

Driven by the motto From Lab to Market, the team has given a multidisciplinary shape to a deep research activity, following the development of the product from research to production, from patentability to market analysis, with a strict plan of the activities.

Understanding the problem

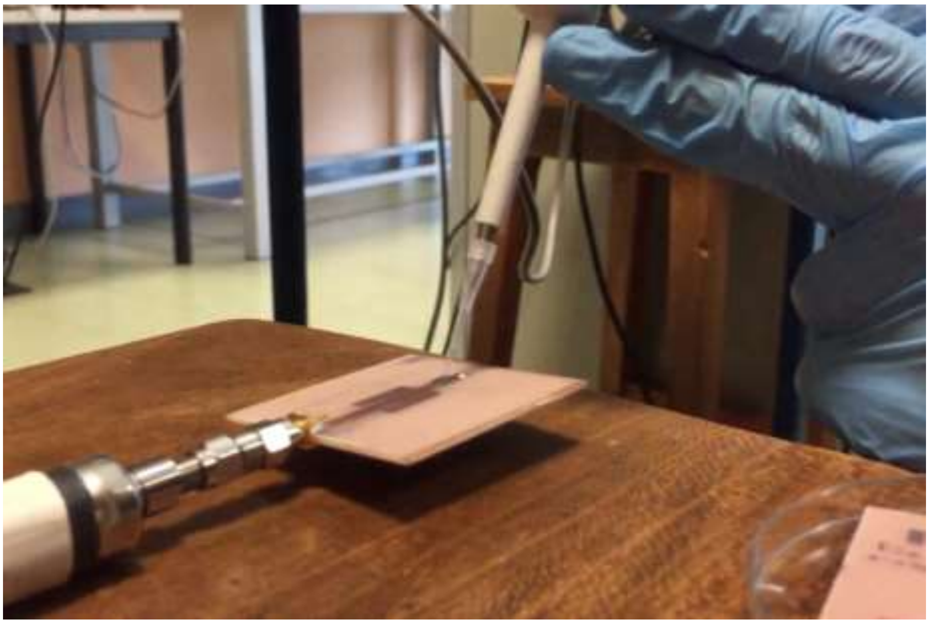
Diabetes is a chronic disease which develops when the pancreas is unable to produce enough insulin (type 1) or when the body cannot use the insulin produced (type 2). It is one of the most widespread diseases across the globe and it is also among the diseases with the fastest growing rate in number of cases. The WHO estimated that more than 422 million people across the globe had diabetes in 2014, in 2012 1.2 million deaths were caused by diabetes, while other 2.3 million happened because of too high levels of blood glucose. From 1980 the number of cases quadruplicated. For these reasons it has been included in the list of the four priority noncommunicable diseases by the World Health Organization (WHO) [1]. High blood glucose has even a wider impact than diabetes, as 3.7 million deaths are related to it. Consequences of diabetes can include loss of vision, cardiovascular events, end-stage renal diseases and lower limb amputations. Monitoring the levels of blood glucose is therefore of paramount importance for early detection and monitoring of the disease.

The most widely used self-monitoring method for blood glucose concentration relies on the fingerpricking approach. It involves sampling blood from a finger via pricking, to be analyzed by test strips and an impedance-based glucometer [2], as shown. It needs to be carried out at multiple intervals throughout the day to help manage elevated glucose levels especially after meals, exercise and dosing of insulin medication. To perform each measurement, the patient is supposed to prick his finger with a needle, allowing the extraction of a small droplet of blood. This may have some drawbacks in the patient life, such as pain, risk of infection of the wound and others.

A possible solution for those issues can be found considering other more accessible biological fluids such as interstitial fluid, ocular fluid, sweat, breath, saliva or urine to perform such kind of measurements.

The main difficulty concerning these alternatives is the lower concentration of glucose to be detected, if compared to blood. To correctly detect the concentration, the sensor requires a lower Limit of Detection (LOD) and higher sensitivity with respect to those implemented for glucose sensing in blood.

According to the standard for evaluating the accuracy of blood glucose meters, called ISO 15197 released in 2013 [3], the accuracy specification for glucose values below 75 mg dl^{-1} requires that the measurement errors lie within ∓15 mg dl^{-1}. Considering that the molar mass of the glucose is 180.16 g mol^{-1}, the minimum detectable variation of concentration must be of 4.16mM. This limits the application of common sensing techniques with other corporal liquids such as sweat or saliva. For this reason, a development of new kind of detectors with higher sensitivity is required to be able to perform non-invasive measurement of glucose concentration in human blood.

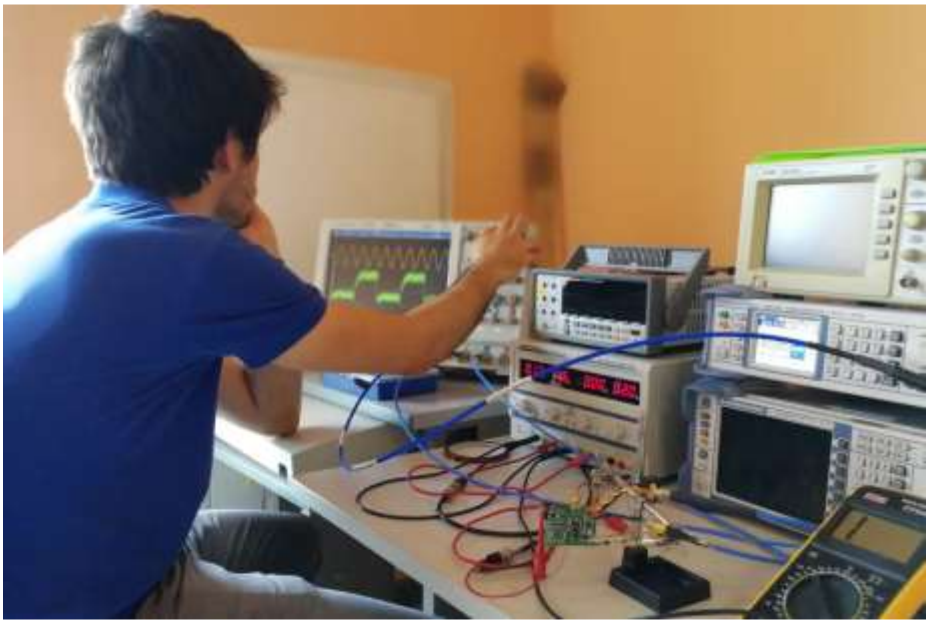


Making of the sensor: deposition of the active graphene film.

Exploring the opportunities

Glucose biosensors are devices adopted in multiple contexts and their features must be tuned on the usage required. Indeed, such devices can either be used in a hospital, by qualified professionals (e.g. nurses) on the patients, or by patients themselves at home. While for the former usage the time required for the sensing is a critical parameter, for the latter simplicity of use and portability are more relevant. Having acknowledged that different situations require different designs, the team proceeded to investigate the crucial features for each one and how companies of the sector are having their products evolving in these directions.

The first step of the analysis consists in acknowledging the stakeholders of the sector, to clarify the needs for the project itself and define the features that the device should comprehend.



Testing of the electronic instrumentation.

The first stakeholders of a glucose biosensor are certainly the end-users, i.e. the patients, which can be either active, measuring their own glucose levels, or passive, needing a third individual such as a nurse or a physician for having their glycemia measured.

Due to their participation, also health operators belong to the list of the project stakeholders. Clearly, companies and businesses producing the devices are relevant stakeholders and will manifest different kinds of needs.

To investigate the needs of each of the involved stakeholders, the decision to prepare and distribute a survey was considered. The survey, that was realized in both Italian and English versions to cover a wider audience, is such that a different flow of questions is followed depending on the category the respondent belongs to.

The survey was mostly distributed online to have a heterogeneous group of respondents. More than 120 answers were recorded before the survey was closed for data analysis.

The four different categories of stakeholders expressed different needs towards the device. In particular, active users require the device to be portable, cheap, fast and easy to use, while passive users are more concerned with comfort. When it comes to health operators the most important requirements become accuracy and durability, while producers have mainly to deal with the costs related to the manufacturing of the device.

As it concerns the present market situation, blood glucose monitoring is a standard sector in which all major pharmaceutical companies invest conspicuous resources to obtain a product suitable for the largest slice of customers. As a result, up to now more than 80 different devices are available on the market, with prices ranging from 9 to 150 $ (most of the devices have a price of around 30-40 $).

Leading players in the field are major companies in the health care area, which have launched the products with the broader diffusion.

However, recent studies have evidenced that the low accuracy of these devices is their main weakness. This limit, which is due to the sensing technique implemented, is also the reason why blood is still the reference fluid, even though everyone acknowledges the discomfort deriving from finger-pricking.

Based on this information, it emerges that a new sensing technology providing a higher accuracy and avoiding finger-pricking, has the potentiality to be disrupting.

Dismissing blood sampling and opting for different body fluids, such as tears, sweat or saliva, may constitute a breakthrough for the quality of life of the patients.

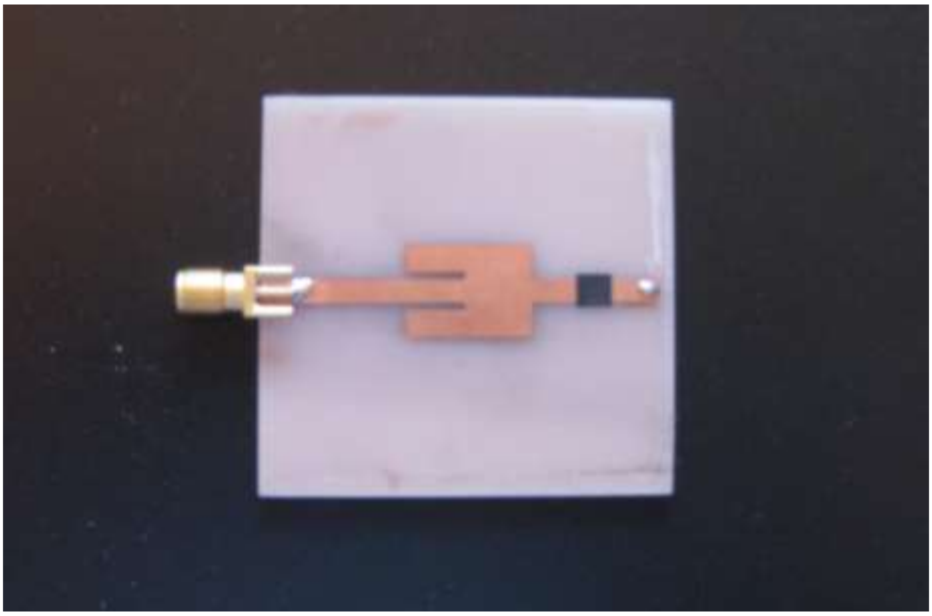
Generating a solution

In recent years, the evolution in research and development in the sector has sparkled and novel designs, with innovative features and materials have been proposed. During the first phase of the project a comprehensive study of the State of the Art in the field of glucose sensing have been performed. Results shows that none of the currently implemented solutions can accomplish all the requirements identified in the previous stage.

Differently, the research lead by Prof. Savi and her research group at Politecnico di Torino on glucose sensors offered a good starting point to create an effective technology to reach the goal of the project. To proceed in the development of the technology, a great effort has been focused on the improvement of the sensor working principle, in particular, the interaction with glucose molecules.

The final sensor design includes a custom-made resonating transducer unit, upon which a functionalized layer of graphene, able to interact with dissolved glucose, is deposited. In particular, the transducing solution is given by a radio frequency resonator (a microstrip patch antenna) able to convert the biorecognition output into a measurable resonance frequency shift. The selected final functionalization strategy is given by a generalized analyte binding approach, where the active biomolecules are GBP (that conformationally binds glucose) or GOx (that catalyzes the oxidation of glucose and is therefore influenced by near-field effects). These molecules are covalently bonded to graphene by means of an optimized binding protocol, which was developed in collaboration with the DISAT Department of Politecnico di Torino.

To qualitatively assess the performances of both setups, a series of equivalent experiments were performed: Raman spectroscopy was executed at Istituto Italiano di Tecnologia (GE) to verify the efficacy of the binding protocol and S11 measurements were carried out at Politecnico di Torino to ascertain the presence and correlation of a frequency shift. Throughout these experiments, the glucose-substrate interactions were mimicked by biomolecules with very similar activity to GBP and GOx.



Top view of the sensor: the microstrip patch antenna is deposed over the square substrate. The dark element on the right is the active graphene film.

During the development of the project, the team has come across the need of a simple way to test the different sensor samples we created. In parallel to the fabrication of the sensing units, RaFrBio team therefore decided to develop a custom testing instrumentation that could allow any user, also those lacking a proper technical background, to easily read the amount of glucose poured onto the sensing element and perform some simple data processing, either on-board or on a PC. In response to a detailed requirements assessment, a real-time, multi-channel measurement setup was built through a complete in-house manufacturing process, pursuing limited dimensions, maximum modularity and simplified data readability above all.

The beating heart of the whole instrumentation is the readout electronics, able to perform the actual measurement of the frequency. The two critical and conflicting requirements to fulfil for this module consist in achieving a very high resolution and wide operative range. To accomplish both the requirements, has been developed a piece of hardware implementing an innovative technique to measure the frequency, based on periods-counting. Due to the high frequency at which the sensor operates, namely above 500 MHz, its design had been challenging. Thanks to the collaboration with a small electronic manufacturing company, namely EDC S.r.l., the hardware has been effectively built and tested. Such an opportunity allows to publish this part of the work in form of a scientific paper.

Main bibliographic references

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