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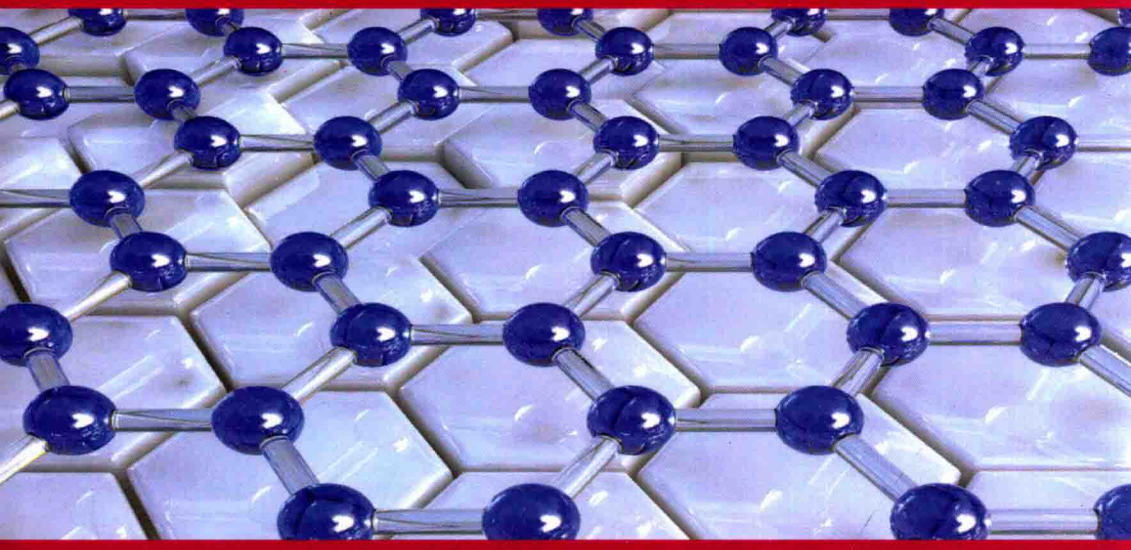

Micro- and Nanoelectromechanical Biosensors

Liviu Nicu and Thierry Leïchlé

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Micro- and Nanoelectromechanical Biosensors

Introduction

In a world where a biological threat may take multiple forms associated with environmental, health or defense issues, the need for versatile biosensing platforms is of vital concern. The variability of biological matter is proportional to the infinite different ways in which it impacts human beings, in timescales ranging from hours (for particularly aggressive viruses such as those provoking hemorrhagic fevers) to years (for somatic evolution processes leading to cancer). Identification and quantification of one or several biological species of harmful potential are the design targets for the vast majority of the existing biosensors.

No matter what type of biological species (be they viruses, bacteria or circulating proteins in the bloodstream) are targeted by a biosensor, the bottom line of the fundamental requirements for a successful biosensing process is always the same: the best specificity, sensitivity and fastest time of analysis. More specifically, we can add portability, user-friendly exploitation interfaces, cost and a few others which are of secondary concern. In a contemporary technological context where the plethora of configurations seems to meet part or all of the previously listed requirements, it seemed of paramount importance to the authors to reassess the basics of exactly what micromechanics can do in order to overtake

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the biosensing area, where compatible. This book intends to shed light upon the field of microelectromechanical systems (MEMS)-based biosensors.

I.1. A brief history of biosensors

In his concise but remarkable review of the field of biosensors, Kissinger [KIS 05] looks back to the early days of biosensing (the 1960s and 1970s) to pinpoint that a "sensor seemed to always be a probe of some sort because of systematic association to pH, ion selectivity or oxygen electrodes". Following the old literature, biosensors are found being called bioelectrodes or enzyme electrodes, or biocatalytic membrane electrodes [ARN 88].

More generally, according to the International Union of Pure and Applied Chemistry (IUPAC) recommendations in 1999, "a biosensor is a self-contained integrated receptor-transducer device, which is capable of providing selective quantitative or semi-quantitative analytical information using a biological recognition element".

The critical feature of the biosensor relates to the selectivity for the specific target analyte; this feature directly impacts the specificity or the process of maintaining the selectivity in the presence of other, potential interfering species. The combination of these quality criteria with miniaturization, low cost and essentially real-time measurements in various fields has generated intense commercial interest.

The last 30 years have witnessed an extraordinary growth in research on sensors in general and biosensors in particular. As underlined by Collings and Caruso in their exhaustive review on biosensor advances [COL 97], "an intensively competitive research area is the result of the combined pressure from the traditional well-springs of

research and development – science push and market pull". The growth rate of research activities on biosensors is shown in Figure I.1.

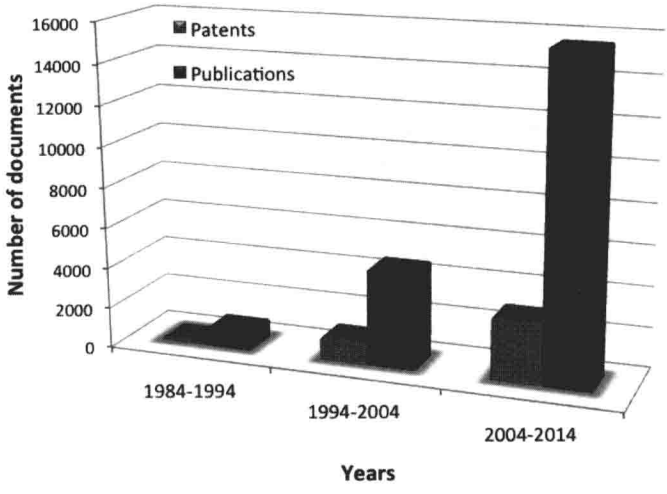

Figure I.1. *Overview of the growth rate of research activities on biosensors since 1984 (Sources: World Intellectual Property Organization database (for patents) and Web of Science Thomson Reuters' database (for publications))*

In spite of all this, there is only one truly commercially successful biosensor: the blood glucose meter for people with diabetes. It is important to note that the glucose biosensor uses the technology that was developed by Clark and Lyons well over 50 years ago and only recently has the public benefited from the potential of such a biosensor. The blood glucose meter is a handheld biosensor based on electrochemical transduction technology [ORA 03] that is produced and commercialized by many companies [TUR 99]. However, in terms of laboratory-based instrumentation, an optical detection system seems to be more commercially viable. Companies such as Affymetrix and Agilent have developed various commercial microarray optical detectors and scanners for genomic and proteomic analysis. Optical

sensors that employ surface plasmon resonance (SPR) detection have also been successfully used in many laboratories and universities [RIC 03]. Hence, commercially available optical bench-size immunosensor systems such as BIAcore™ (Biacore AB, Uppsala, Sweden) and IAsys (Affinity Sensors, Cambridge, UK) have found their market in research laboratories for the detection and evaluation of biomolecular interactions.

Still, the development of disposable sensors in conjunction with handheld devices for point of care measurements has featured prominently. Microfabrication technology has played an important part in achieving miniaturized biosensors. Such technology has provided cheap, mass-producible and easy-to-use/disposable sensor strips. Similarly, electrochemical methods have played a pivotal role in detecting the changes that occur during a biorecognition event, and the merging of microfabrication with electrochemical detection has led to the development of various handheld biosensor devices. In fact, i-STAT has developed the world's first handheld device for point-of-care clinical assaying of blood (Figure I.2), noting that this biosensor array employs several electrochemical-based transduction methods (i.e. potentiometric, amperometric and conductometric) [PEJ 06]. However, this is the only example demonstrating the power of microfabrication technologies for the development of biosensors with high integration and multiplex analysis capabilities.

Will markets harvest the fruits of the next generation of biosensors? In fact, it is suggested that a major part of research and development (R&D) activity in this area rarely results in a commercial product [FUJ 04]. However, the observed growth in biosensor research increases the probability of witnessing another success story in the next couple of decades. The future R&D outlook for biosensors looks positive despite very little market growth/progress over the past few years.

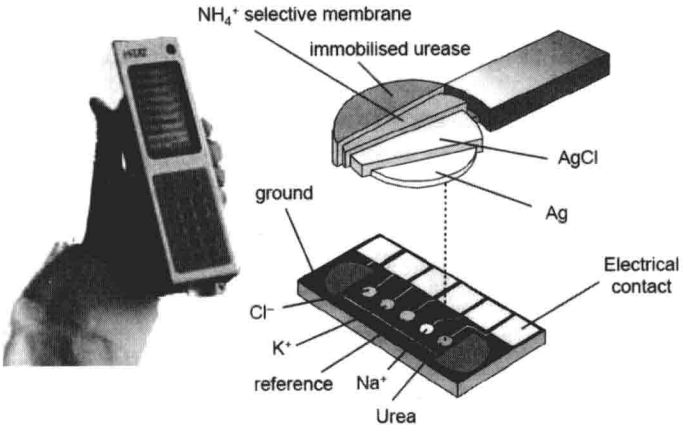

Figure I.2. *The i-STAT multisensor for monitoring various blood electrolytes, gases and metabolites (www.abottpointofcare.com)*

I.2. What is biosensing?

To introduce the field of MEMS biosensors, the concepts and terminology that will be discussed in the next sections and chapters of this book are first to be clarified and described.

I.2.1. *Definitions*

Biosensing: this term is used when a "search and quantify" cycle of operations for one or more biological species (proteins, viruses, bacteria, etc.) is conducted, starting from a sample (either in a gaseous, liquid or solid state) and making use of analytical means of variable complexity.

Biosensor: this is a biosensing device or system made up of two fundamental components: a functionalized solid surface and a transducer which, in turn, transforms a biological reaction (or biological recognition event) taking

place on the functionalized surface into a measurable physical signal (Figure I.3).

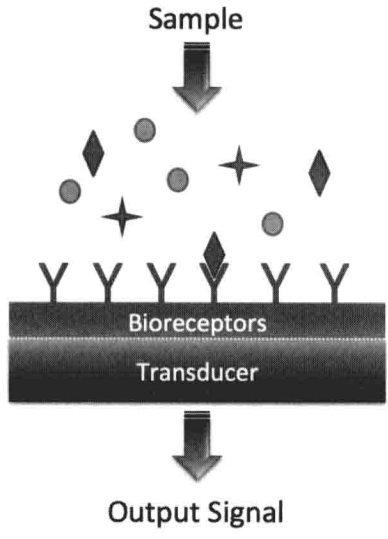

Figure I.3. *Principle and main components of a biosensor*

MEMS biosensor: this is a biosensor using a microelectromechanical system as a transducer.

Multiplexed biosensing: this consists of detecting/dosing several different kinds of biological species present in the same sample, at the same time, in the same fluidic chamber by means of an array of biosensors that are deterministically functionalized prior to the contact with the sample.

Functionalization: this is a succession of chemical and biological reactions on a solid surface, aiming to provide specific reactivity with biological species that are the final targets of a biosensing operation. Functionalization can be either deterministic (i.e. precisely localized) or arbitrary (i.e.

the whole functionalized surface will bear the same chemical and/or biological functionalities).

Fluidics: this is a set of techniques enabling fluid sample circulation toward one or more biosensors. Except for specific requirements, the fluidics operated in most practical cases is "basic fluidics", meaning a reduced volume (several milli-/microliters) closed chamber (or reservoir) made up of biocompatible material (glass, plastic or biologically friendly metals) and bearing access ways (inlets and outlets) which ensure the contact with the outside world by means of flexible capillary tubes.

Sample preparation: this consists of a sequence of steps carried out before sample analysis and aims to render a raw biological sample in a solid, liquid or gaseous phase (e.g. food, blood and air sample) appropriate for biosensing. A clean sample is often required when using sensing techniques that are not responsive to the analyte in its *in situ* form or when the measurement results are distorted by interfering species. Sample preparation includes filtering and separation of unwanted entities (particles, biological entities or chemical species), and dissolution or preconcentration and isolation of the target analyte within an appropriate diluent using various techniques.

I.2.2. *Important numbers and characteristics*

For a biosensor to be a "meet-all-expectations" device, it must fulfill a set of requirements (also known as specifications) that are specific to the biosensing domain. The most important of them are defined as follows:

Sensitivity: this is the transducer output variation induced by the biological interaction events happening on its functionalized surface.

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Limit of detectable output (LDO): this indicates the ultimate performance in terms of discernibility of the transducer's output variations with respect to the measurement noise.

Response time: this relates to the time period between the sample injection in the fluidics surrounding the biosensor and the moment when the transducer's output signal is stabilized after having shifted because of the biological interactions effects happening on its functionalized surface.

The list of performances given above is far from being exhaustive. Other specifications exclusively dependent on the format of the transducer might be given. However, we deliberately limit the enumeration to the most generic features that have to be kept in mind and addressed before aiming to use MEMS as biosensors. For an exhaustive classification of biosensors and their associated specifications, readers are referred to [GIZ 02].

I.2.3. *Classification of biosensors*

Biosensors can be classified according to three factors: (1) the type of receptor (e.g. an immunosensor), (2) the physics of the transduction process (e.g. an amperometric sensor) or (3) the application (e.g. a medical biosensor).

The biological recognition element or bioreceptor is a crucial component of the biosensor device. Generally, we can draw three principal classes of biosensors that are distinguished from each other by the nature of the biological process and by the involved biochemical or biological components, i.e. biocatalytic (e.g. enzyme), immunological (e.g. antibody) and nucleic acid (e.g. deoxyribonucleic acid – DNA).

The transducer is another component of the biosensor, which plays an important role in the detection process. A wide variety of transducer methods have been developed in

the past decade; however, recent literature reviews have shown that the most popular and common methods presently available are (1) electrochemical, (2) optical, (3) piezoelectric, and (4) thermal or calorimetric [HAL 90, BUE 93, GIZ 02].

These groups can be further divided into general categories: non-labeled or label-free types, which are based on the direct measurement of a phenomenon occurring during the biochemical reactions on a transducer surface, and labeled, which relies on the detection of a specific label that is linked to the analyte to detect. Research into label-free biosensors continues to grow [COO 03]. However, biosensors based on the detection of labeled analytes are more common and are extremely successful in a number of platforms (especially those based on the use of fluorescent labels detected by optical means).

From an application point of view, even if medical and clinical applications are the most lucrative and important avenues for biosensors, other areas like environment, industrial process monitoring and control, or defense require specific biosensing systems. Moreover, commercial biosensors can be divided into two categories on the basis of whether they are laboratory or portable/field devices.

I.3. Biosensing applications and examples

To better assess the impact that MEMS might have on the biosensing domain in the next few years, let us use three fictive examples of potential applications, respectively, in the environmental, health and defense realms. The enumeration of requirements related to real-world situations will allow us to emphasize the advantages of such devices with respect to alternative, already existing solutions. Each of the chosen examples will be presented following the same synoptic basis: (1) context of the raised biological issue and (2) bioMEMS-based optional solution to avoid the bio-crisis. A

general conclusion will highlight the added value brought by the bioMEMS solution.

I.3.1. *Environment*

I.3.1.1. *Context of the raised biological issue*

It had been several days since the local university canoeing team set up its training camp on the border of the Blue River, the same as every year, in early summer time, before starting the competition season. This year, the training camp had gathered together the whole team, meaning there were 24 athletes, and 3 trainers. Despite there having been good weather conditions since the beginning of the stay, by the middle of the second week three of the young athletes had started to show signs of evident illness such as fever, chills, myalgia and intense headaches. It mostly looked like a flu-related syndrome, which was all but probable at this time of year.

The decision to break camp and return home was rapidly taken to allow immediate identification of the origins of the illness and to seek treatment as soon as possible. Finally, it took 8 more days (and 6 more infected students) to isolate the cause: leptospirosis, a disease caused by infection with the Leptospira bacteria which is transmitted from animals to people when water contaminated by animal urine comes into contact with the eyes or with the mucous membrane.

I.3.1.2. *How could all this have been avoided?*

Let us imagine the same canoeing team setting up the camp on the Blue River border. Each and every morning, one hour before starting the training day, the same ritual is taking place on the river banks: one of the trainers is pouring few milliliters of water over a small, portable, handheld device. The liquid is driven through a network of flexible capillary tubes while being filtered such that it

becomes an inorganic debris-free sample, ready to be analyzed by an array of several dozens of tiny mechanical sensors functionalized with antibodies specific to the most common biological pathogens living in dirty, infected water.

The analysis result is provided within a couple of minutes: if it is positive to one or several pathogens, the measurement protocol is repeated once to avoid a false positive conclusion and if confirmed, the camp is eventually evacuated, thus avoiding potentially infectious exposure of the team and subsequent illness.

I.3.2. *Health*

I.3.2.1. *Context of the raised biological issue*

The same as every day since his wife had been gone, Mr. Jones, 92 years old, had started that morning by making a pot of coffee for breakfast. He was one among the lucky elderly whose autonomy was preserved by a satisfactory overall physical condition providing regular, basic analysis to monitor his health condition. But that morning, unlike the previous ones, Mr. Jones felt like something had gone wrong: a sort of squeeze-like sensation located at the center of his chest was bothering him. Trying not to pay much attention to it, Mr. Jones sat for a few moments on a seat in his kitchen hoping that the chest pain was about to vanish. Instead, his vision became blurred and sweat started to pearl on his forehead. What if the warning was more than serious? He finally grabbed his phone and dialed the emergency number trying to keep calm so he could repeat his symptoms to the operator in a clear manner. Suspecting a heart attack, a medical team was immediately directed to Mr. Jones's address, and half an hour later the primary care protocol was applied. While ensuring that Mr. Jones's airway was normal and it was not laborious for him to breathe, the first results delivered by a handheld electrocardiograph showed that the heart was functioning well. The severe heart attack

hypothesis had for the moment been swept away even if cardiac biomarker monitoring had to be done over several hours at the hospital.

Realizing that his heart was finally performing well, Mr. Jones started to feel better and progressively told the medical team about the nightmares he had the night before: he had dreamt of his wife fighting hard against the cancer a few years before and crying for help while he could not do anything. The medical team members started to understand the origins of Mr. Jones's panic and while they kept reassuring him that everything was under control, they decided to transfer him to the hospital for supplementary tests in order to definitely avoid any doubt.

I.3.2.2. *How could all this have been avoided?*

Imagine Mr. Jones at the very beginning of his symptoms of pain and panic grabbing a small suitcase while having a seat in his kitchen. The suitcase had been given to him by his family doctor a few months before and he had been trained to use it in the case of a cardiac alert. Two main elements were packed inside the suitcase: a small, flexible patch to be applied onto the chest and a smartphone-like device having been calibrated to identify and measure cardiac biomarkers (specific to cardiac disorders) in a small quantity of a blood sample. The instructions for use were simple: first, apply the flexible patch to the chest and push an ON/OFF switch, and second, prick one finger and transfer one drop of blood onto a red-colored area of the smartphone-like device, then wait.

While the flexible patch was transmitting, in real time, the electrocardiogram signals to a remote emergency medical team, the main cardiac biomarkers were analyzed by means of an array of specifically functionalized bioMEMS sensors embedded in the smartphone-like device and the results progressively delivered to the same emergency unit. This

first emergency assessment would eventually help the remote medical team in the decision-making process, i.e. to send a doctor to perform further investigations rather than sending a whole medical team in a situation that does not necessarily require such an intervention.

I.3.3. *Defense alert*

I.3.3.1. *Context of the raised biological issue*

The Airbus 380, flight AF066, carrying more than 500 passengers from Paris to Los Angeles has been waiting for landing approval since 1 pm and the risk of the carrier running out of kerosene is greatly increasing minute after minute. The reason for the delay is critical; a group of three people took control of the plane several hours before the scheduled landing time and since then they have been seriously threatening to spread unknown, airborne biological agents inside the cabin if their demands are not met immediately. They have requested the liberation of one of their associates who will board the airplane, and after refueling they have demanded permission to take off for a new destination. To prove their resolve, the three men have isolated several passengers on the upper deck and have exposed them to an aerosolized product. Shortly after exposure, almost all of them presented disturbing symptoms like violent nausea and headaches.

A decision was rapidly taken to follow the terrorists' demands and their requests were completely fulfilled. Three days later, the story ended in the liberation of all the hostages on the tarmac of an eastern African airport when it rapidly transpired that the "bio-aggression" had been completely fake. In reality, the three men had used a vomiting aerosol based on chloropicrin which helped creating the illusion of symptoms related to particularly aggressive biological agents spread among the chosen passengers.

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