Cantilever Biosensors

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ABSTRACT

An increasing number of recent reports confirm the potential of MC sensors for environmental and biomedical applications, and the multifaceted functionality of MCs indicates their uniqueness as compared with more traditional sensor designs. Unlike many other types of transducers for chemical sensors, MCs are simple mechanical devices. They are tiny plates or leaf springs, typically 0.2-1 ìm thick, 20-100 ìm wide, and 100-500 ìm long, which are connected on one end to an appropriate support for convenient handling. This article only seeks to make a specialized comment on the latest applications of cantilever biosensors in the field of Biological, Physical and Chemical Sciences. Likewise, the reader is also provided with some of the potentials of these biosensors which offer detailed information on the specific molecular interactions. In addition, reference is made to the principle of functioning, achievements and tendencies of this efficient tool.

Key words: biosensor, cantilever, electromechanical, molecular interactions

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RESUMEN

Biosensores Cantilever. Numerosos artículos recientes confirman el potencial de los biosensores microcantilever para aplicaciones ambientales y biomédicas, así como su funcionalidad multifacética, lo cual indica su singularidad comparada con los diseños de los sensores más tradicionales. A diferencia de muchos tipos de transductores para sensores químicos, los microcantilever son simples dispositivos mecánicos: diminutas vigas o resortes batientes, cuyas dimensiones son: grosor entre 0.2 y 1 μm, ancho: entre 20 y 100 μm, y largo: entre 100 y 500 μm. Ellos están conectados al extremo de un soporte apropiado para su conveniente manipulación. El presente artículo es un comentario especializado sobre las más recientes aplicaciones de los biosensores cantilever en el campo de las ciencias biológicas, físicas y químicas. También se presentan al lector algunas de las potencialidades de estos biosensores que proporcionan información detallada sobre interacciones moleculares específicas. Además, se hace referencia al principio de funcionamiento, los logros y las tendencias de esta eficaz herramienta.

Palabras claves: biosensor, cantilever, electromecánico, interacciones moleculares

Introduction

Most conventional micro and nanoelectromechanical systems (MEMS and NEMS, respectively) are designed for detecting and sensing. The sensing principle varies according to the device, the nature of the analyte molecules, and the precision required. Capacitance, piezoresistance and resonance frequency are among the sensing principles depending upon the mechanical properties of the device.

Micromachined cantilevers were first used as force probes in Atomic Force Microscopy (AFM). Their extreme sensitivity to several environmental factors, such as noise, temperature, humidity and pressure was immediately evident. In 1994, research teams in Oak Ridge National Laboratory and IBM, converted the mechanism causing interference into a platform for a novel family of biosensors. They discovered that a standard MFA cantilever could function as a microcalorimeter, with sensitivity in the 10^{-15} Joule range, and a substantial improvement as compared to traditional techniques. By measuring the change in resonance frequency, microcantilevers were shown to be sensitive to mass changes, with a better yield than piezoelectric gravimetric conventio-nal sensors. The laboratories with MFA instruments displayed a substantial interest in cantilevers as a new platform for a variety of chemical and physical biosensors.

Recent advances in microfabrication techniques have boosted the discovery of novel applications for micro and nanotools. Micro and nanocantilevers have been used as a new class of biosensors: they recognize proteins with exquisite sensitivity and can detect small amounts of materials, especially pathogen bacteria. This is a very useful property for medical diagnosis and food control. Directed micro and nanoelectromechanical devices, are also useful as highly sensitive and immunospecific multifunctional biological detectors.

Cantilever

Cantilevers (springboard) are nanomechanical biosensors, microfabricated with the standard silicon technology. Their sizes are in the micrometer or nanometer ranges (Figures 1 and 2). Due to their intrinsic flexibility, together with the availability of techniques designed to monitor bending, cantilevers have become versatile tools for SF

This technology is already established and well documented as a multifunctional and highly sensitive technique, and a real time method useful for a variety of applications, such as plastic explosive detection using gas biosensors, whole microorganism detection as part of liquid biosensors, or DNA and proteins studies.

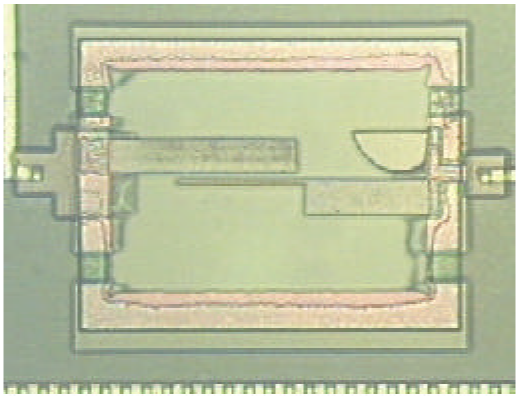

Figure 1. Nanoresonator for Nanomass project.

Configuration of the measurements

By incorporating a piezoresistor to each cantilever in a Wheatstone bridge type configuration, it is possible to read resistance changes as voltage changes. The Wheatstone bridge configuration uses a pair of cantilevers; one of them will be used as reference. The differential signal between both cantilevers will be the output of this configuration. The signal-noise relation is substantially improved with this configuration, and the noise originated by unspecific binding, thermal fluctuation, or vibrations is eliminated.

Non-specific binding to the surface is a general problem that must be minimized in all analyses. Although the complete elimination of this parameter is not possible, its influence on detection could be controlled with the use of the reference cantilever.

Functioning principle: detection of molecular interactions

The immobilization of molecules on the cantilever surface is required for its use as a nanomechanical sensor (Figures 3 and 4). The selection of the molecule depends on de intended application. This principle applies whether if the molecule to be detected (analyte) will be presented in a liquid or a gas phase. The immobilized molecules provide the cantilever with specificity for the analyte. The specific molecular interactions taking place at the flexible surface of cantilever increase surface tension, forcing the cantilever to bend [1]. This type of surface tension induced by molecular interactions is not generally observed on the surface of common materials. The cantilever senses the tension and bends in response to the free energy changes taking place at its surface.

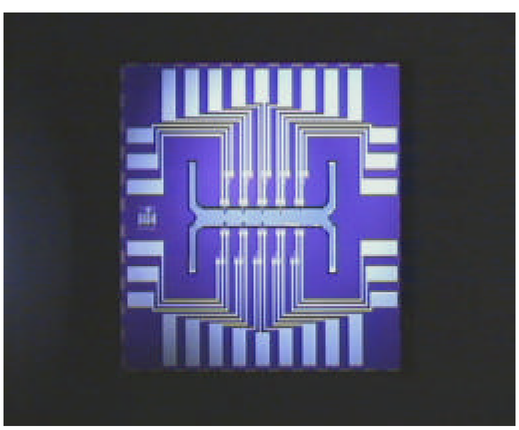

Figure 2. A ten microcantilever array integrated in a micro-channel.

Electrical readout

The «optical lever» technique has been traditionally used to monitor the bending of the cantilever. This technique was originated in Scanning Probe Microscopy. Cantilever sensors equipped with integrated piezoresistors have been recently developed. Those biosensors can carry out a full electrical readout of the cantilever bending. This eliminates the need for complex aligning of laser, allows the measurements of turbid liquids, and reduces the cost of instrumentation.

By using the principle of electric readout it is possible to create portable devices to conduct decentralized analyses, avoiding the transportation of the samples to a central laboratory.

The integrated piezoresistor is a sensing element enabling the measurement of cantilever bending, below the nanometrical scale. Its readouts change when a force is applied. It is generally difficult to get the piezoresistive readout of liquid samples with the cantilever, due to the separation between the piezoresistor and the liquid.

The information obtained

The application of nanomechanical cantilevers to the analyses of molecular interactions can provide very detailed information. The interaction occurs at the surface of cantilever where one of the interacting molecules has been immobilized. The exposure of the *chip* to one or more analytes, which specifically binds to the immobilized molecule, provides the following information:

1. Cantilever technology. 2006.[Sitio en Internet]. Disponible en: http://www.Cantion.com/cantilever_technology.htm. Último acceso:12 octubre 2006.

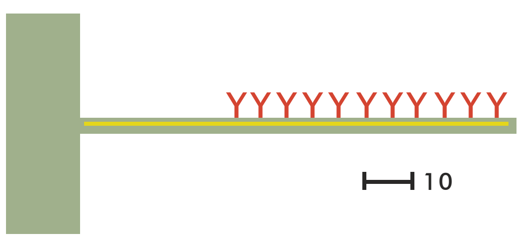

Figure 3. Cantilever (with integrated piezoresistor) without detection.

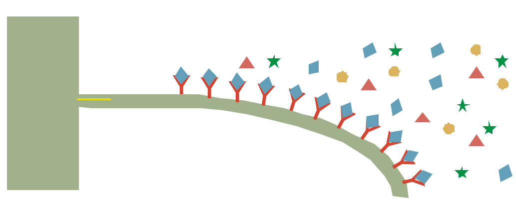

Figure 4. Cantilever with detection. Presence of molecules immobilized at the surface.

The concentration of the analyte

The signal of the cantilever at the stationary estate and/or the index of surface binding are proportional to the concentration of the analyte. The solutions can be prepared with purified molecules or, alternatively, the analyte can be a part of a complex mix.

The kinetics of the interactions

Through real time monitoring of a molecular interaction it is possible to calculate its speed. Once the sample replaces the buffer solution into the flow cell, the analyte molecules bind to their «immobilized partner» at the cantilever surface. When the sample is washed out the flow cell and replaced by the buffer solution, the attached molecules will start to dissociate from their «immobilized partner».

As in many analytical techniques, the selectivity, dynamic range, and other functional characteristics of the assay are mostly determined by the selection of the coating molecule.

Signal Measurement

The differential voltage readout from the Wheatstone bridge is monitored in real time. A specific interaction at the cantilever surface occurs when the sample is loaded into the sensor. The corresponding change in the surface tension will bend the cantilever. The progress of the interaction is then quantitatively determined by plotting the time course of the response. The amplitude and reaction time before a new steady state is achieved provide information on analyte concentration and the kinetics of the interaction.

Applications

The surface of the cantilever should be coated with a layer of detector molecules that can react with the analyte and detect biochemical reactions at its surface.

This novel detection technique based on cantilevers is extremely sensitive. Cantilever based biosensors have enormous potential, especially in the field of biochemical analyses. Fast and simple biochemical detectors based on this detection method can be constructed. Studies of simple molecular interactions would also be feasible due to the exquisite mechanical sensitivity of micro-cantilever (Figure 5).

The technology of cantilever biosensors is applicable to a number of specific tasks [2]:

1. Life Sciences: For studying the bases of the interaction among biological molecules. To develop novel analyses based on this platform with a potential applicability to portable devices.

2. *In vitro* diagnostics*:* To develop faster, more sensitive and label free methods (no tracers are needed for final identification) to analyze chemical or biological samples.

3. Drug discovery: To study the interactions between small molecules and their specific receptors. To conduct multiplexed analyses increasing the parallelism and contents of the results.

4. Fresh water control: To detect heavy metal ions in fresh water. To develop assays that could be useful to implement a decentralized system to monitor the quality of the resins, or other chemicals used in the fresh water distribution system [3].

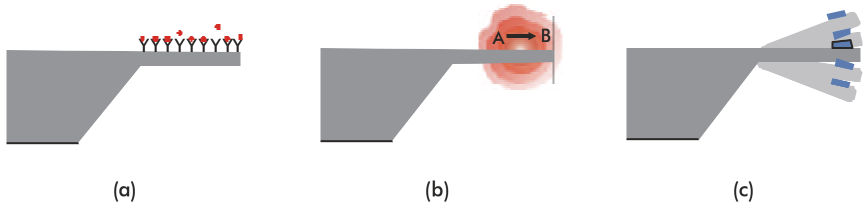

Figure 5. Application of cantilever to the detection of superficial tension changes (a), temperature (b), and mass (c).

Achievements

The advances experienced in nanotechnology have enabled the development of portables systems for biological, physical and chemical applications and made it possible to reach ultrasensitive detection levels with a very low consumption of reagents and analytes.

Mass changes in the order of 1.10^{-12} grams, corresponding to 1 Hz variation in resonance frequency, have been detected by nanomechanical biosensors [4].

A low tension, silicon nitride cantilever sensor has been developed for the detection of *Escherichia coli* (*E. coli*). This sensor is based on the measurement of resonance frequency, and is an example of the application of micromechanical oscillators to single cell detection. The high affinity antibodies used are able to capture a single *E. coli* cell. The binding step consists of the interaction between antibodies specific for *E. coli* O157:H7, immobilized on the cantilever surface, and the O157 antigen displayed at the surface of the pathogen *E. coli* O157:H7. After the specific capture of E coli cells an additional charge is detected by measuring the changes in the resonance frequency of the micromechanical oscillator. By analyzing the spectra of resonance frequency, before and after antigen-antibody binding, the mass of a single *E coli* cell was estimated in 665 fg, a figure which agrees with previous reports [5].

A multidisciplinary group from the University of Aarhus, Denmark, successfully detected the sequence of one DNA strand by using a cantilever with an internal piezoresistive element[6].

The use of the micromechanical and nanomechanical cantilever has facilitated real time studies of molecular interactions, the fast detection of whole bacteria, and the detection of toxins and antibiotic residues in food products. One of the fields with a fast and broad application of nanomechanical biosensors is the detection of contaminant chemicals in food or in the environment.

A simple system for multiple and simultaneous immobilization of molecules in separate sections of cantilever arrays has been developed. This configuration notably the efficiency of any chemical measurement. An additional advantage is to treat the cantilever with the same molecule, because more data can be collected with each measurement, and the results are more reliable [7].

An array configuration of 33 microcantilevers made of a SU-8 polymer has been developed for DNA detection. The mechanical properties of this system, such as the *spring* constant, resonance frequency and the quality factor, are characterized as a function of dimensions and environment. This technology

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3. Nanomechanical cantilever. 2006. [Sitio en Internet]. Disponible en: http://www.Cantion.com/applications.htm. Último acceso:12 octubre 2006.

4. Nanomechanical sensor. 2006. [Sitio en Internet]. Disponible en: http://monet.physik.unibas.ch/nose/inficon/. Último acceso:4 noviembre 2006.

5. Single cell detection with micro-mechanical oscillators. 2006. [Sitio en Internet]. Disponible en: http://scitation.aip.org/getabs/servlet/GetabsServlet?prog=normal&id=JVTBD9000019000006002825000001&idtype= vips&gifs=yes. Último acceso:4 noviembre 2006.

6. Piezoresistive cantilevers. 2006. [Sitio en Internet]. Disponible en: http://cat.inist.fr/?Modele=afficheN&cpsidt=17066108. Último acceso: 4 noviembre 2006.

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exhibited six times better sensitivity as compared to the silicon based commercial cantilever [8].

Multiple applications of nanomechanical biosensors have been reported, among them are: biochemical detection [9], detection of DNA [10], proteins [11], pesticides [12] and TNT explosives [13].

SU-8 cantilever sensor system with a integrated readout

Cantilevers are conventionally built with silicon and use an known optical system from AFM for detection. This novel application uses the SU-8 polymer as a mechanical support, and an integrated force sensor made of gold is used for the readout [14].

Design of an artificial nose

An artificial nose is a portable system that identifies several mists of volatile organic compounds. The analyte is transported by an automatic gas flow system into the analysis chamber containing an array of nanomechanical cantilevers. Data capturing and control is carried out with a personal computer [15].

Conclusions

The field of microtechnology and MEMS has experienced an exponential growth during the last two decades of the XX Century. Researchess have recently focused on the search for alternative materials to the already traditional silicon based technology. One of the most promising materials for the novel micro and nanosystems designs is the SU-8 polymer. Among its useful properties are its photosensitivity, transparency to visible light, a low Young's modulus (also known as elasticity modulus, it is a mechanical parameter describing the degree of rigidity of a solid material), and a high compatibility. This is a material with a high degree of chemical and thermal stability, allowing the construction of very thin microstructures with optimal signal/noise relation. The production process of the cantilever, based on micromachined surfaces, is much more simple, inexpensive and versatile using SU-8. This facilitates the integration of novel functions and the development of a wider range of cantilevers, covering a variety of forms and applications.

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