Nanomechanical biosensors: a new sensing tool

L.G. Carrascosa, M. Moreno, M. Álvarez, L.M. Lechuga

Biosensors based on microcantilevers have become a promising tool for directly detecting biomolecular interactions with great accuracy. Microcantilevers translate molecular recognition of biomolecules into nanomechanical motion that is commonly coupled to an optical or piezoresistive read-out detector system. Biosensors based on cantilevers are a good example of how nanotechnology and biotechnology can go together. High-throughput platforms using arrays of cantilevers have been developed for simultaneous measurement and read-out of hundreds of samples. As a result, many interesting applications have been performed and the first sensor platforms are being commercialized. This review covers the basic working principles and the types of sensor format, the fabrication and the reported applications in chemical and biological analysis, trends in cantilever fabrication, examples of the commercial instrumentation available, and future developments.

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Keywords: Biomolecular interaction; Biosensor; Cantilever; Fabrication; Nanomechanics

1. Introduction

Biosensors are devices that take advantage of the high specificity of biological reactions for detecting target analytes. They couple a biological recognition element (specific to the target analyte) with a physical transducer that translates the bio-recognition event into a measurable effect, such as an electrical signal, an optical emission or a mechanical motion. In the early 1960s, Clark and Lyons [1] and Updike and Hicks [2] developed the first biosensor, based on the specific catalytic interaction of the glucose oxidase enzyme with glucose. Since then, there has been rapid growth in research activities in this area and the biosensor field has made great advances in developing new sensing devices capable of characterizing and quantifying biomolecules in many fields, such as biomedical, industrial or environmental control.

However, besides the excellent results obtained with existing sensor technologies, we still need biosensors able to detect, in a direct way, very low (picomolar to femtomolar) levels of a great number of chemical and biochemical substances in areas such as environmental monitoring, industrial and food processing, healthcare, biomedical technology, and clinical analysis. Progress in microtechnologies and nanotechnologies allows development of highly sensitive sensors with the additional advantage of miniaturization [3]. Microbiosensor or nanobiosensor devices based on microelectronics and related micro-electromechanical system (MEMS) technologies provide devices that could easily be integrated into portable “lab-on-chip” platforms to perform “point-of-care” analysis because, for many applications, portability is a major issue. Additional advantages of this approach to fabrication are robustness, reliability, low energy consumption, and mass production with consequent reduction in costs.

Microcantilever sensors are most promising for microbiosensors and nanobiosensors. This new class of highly sensitivity biosensors can perform local, high resolution and label-free molecular recognition measurements [4,5]. They are derived from the microfabricated cantilevers used in atomic force microscopy (AFM) and are based on the bending induced in the cantilever when, for example, a biomolecular interaction takes place on one of its surfaces. The microcantilevers translate the molecular recognition of biomolecules into nanomechanical motion [6] (from a few nm to hundreds of nm), which is commonly coupled to an optical or piezoresistive read-out system [3,7,8]. Research in this new type of sensor is growing exponentially after the landmark paper of Fritz et al. in 2000, in which they showed the ability of microcantilever sensors in discerning single-base variations in DNA strands without using fluorescent labels.
This paper made a deep impression on the biotechnology field and marked the beginning of a major research effort on this field. Shortly afterwards, microcantilever sensors were also shown to work in DNA hybridization [4,9] and detection of proteins involved in cancer [10] and other diseases [5,11] with increased accuracy, as well as in environmental sciences [12]. Cantilever sensors have also been used for detecting chemicals, such as volatile compounds [13], warfare pathogens [14], explosives [15], and glucose [16], and ionic species, such as calcium ions [17].

Microcantilevers are typically made of silicon/silicon nitride or polymer materials, with dimensions ranging from tens to hundred of μm long, some tens of μm wide and hundreds of nm thick. Moreover, these devices can be fabricated in arrays comprising 10 to thousands of microcantilevers, so they are a promising alternative to current DNA and protein chips because they could permit parallel, fast, real-time monitoring of thousands of analytes (e.g., proteins, pathogens, and DNA strands) without any need for labeling. When fabricated at the nanoscale (nanocantilevers), the sensitivity goes down and expected limits of detection (LODs) are in the femtomole (fmol) to attomole (amol) range with the astonishing possibility of detection at the single-molecule level in real time [18].

In this review, we will focus on describing recent developments in nanomechanical sensors, starting with the working principle and the main read-out systems, and covering all aspects related to fabrication, implementation and the main applications. Finally, we will discuss the future prospects for this exciting new technology.

2. Microcantilevers as transducer elements

Microcantilevers can transduce a number of different phenomena, such as changes of mass, temperature, heat, or stress, into bending (static mode) or a change in resonant frequency (dynamic mode) [7], which can be monitored.

Adsorption of molecules, when they are restricted to one of the cantilever surfaces, produces differential surface stress that bends the cantilever. At the same time, the resonant frequency of the cantilever also varies due to mass loading. The bending and the changes in resonant frequency can be monitored by several techniques, with optical beam deflection, piezoresistivity, piezoelectricity, interferometry, capacitance, and electron tunneling among the most important [3]. Changes in resonant frequency can be detected by measuring the thermal noise of the cantilever. However, to achieve great sensitivity, especially when working in liquids, it is necessary to pre-energize the cantilevers by using alternating electric, magnetic, or acoustic fields.

The key to using microcantilevers for selective detection of molecules is the ability to functionalize one surface of the silicon microcantilever in such a way that a given target molecule will be preferentially bound to that surface upon its exposure. The sensitivity of detection can therefore be greatly enhanced by applying an appropriate coating to one cantilever surface (see Fig. 1 for details). This strategy allows microcantilever sensors to measure extremely small changes due to molecular adsorption and, for that reason, they are extremely sensitive biosensors; with the cantilever technique, it is possible to detect surface stress as small as about \(10^{-4} \text{ N/m}\). Such measurement is also quantitative, related to the concentration of the analyte being detected [6].

Nonetheless, the factors and the phenomena responsible for the surface-stress response during molecular recognition remain unclear. Several factors are thought to be involved, but they are the subject of great controversy in the scientific community [4,19–21]. Electrostatic interaction between neighboring adsorbates, changes in surface hydrophobicity and conformational changes of the adsorbed molecules can all induce stresses, which may compete with each other and mean that the change in stress is not directly related to the receptor-ligand binding energy. This is particularly the case for biological adsorption, due to the complexity of the interactions involved.

3. The most common read-out schemes

A scheme for signal read-out is critical for real-time measurement, accuracy and the possibility of integrating microcantilever biosensors, so it is crucial to implement a read-out system capable of monitoring changes with sub-nm accuracy. Static and dynamic detection methods have proved to be very sensitive when working in air; however, when operated in liquids, both the resonance peak and the quality shift toward much lower values than in air due to the damping effect of the liquid. This
factor dramatically affects measurements based on the dynamic mode, making this method less suitable for monitoring biochemical processes in aqueous environments than the static mode. For this reason, as biological reactions take place in liquids, microcantilever sensors operating in the static mode are especially suitable as a platform for performing nanomechanical biomolecular assays. There are a few demonstrations of biomolecular interaction detection using the resonant frequency method [22].

As was mentioned previously, several techniques have been employed for detecting the bending of the cantilever but the optical and the piezo-resistive are the most popular and are compatible with array formats. Moreover, under real conditions, sensors have to be stable in the long-term, and selective and sensitive to the target molecule with no cross-talk reactions. Non-specific binding of molecules and noise sources, such as vibrations and temperature changes, can be avoided using differential measurements with a passivated cantilever as reference.

3.1. Optical read-out
Optical read-out is one of the most common schemes for detecting the movement of microcantilevers, as derived from standard AFM. The displacement of the free end of the cantilever is measured using the optical deflection of an incident laser beam on a position-sensitive photodetector, which allows the absolute value of the cantilever displacement to be calculated (see Fig. 2(A) for details). This method provides sub-angstrom (sub-Å) resolution and can be implemented easily.

The main disadvantages of this read-out technique are that it requires external devices for deflection measurements, so that their continuous alignment and calibration are very time consuming. Optical techniques are sensitive to changes in the optical density of the sample and can also be subjected to artifacts, due to changes in the optical properties of the medium surrounding the cantilever, and that can move the laser spot on the photodetector surface. In addition, implementation of an optical method for read-out of arrays is technologically challenging, as it requires an array of laser sources with the same number of elements as the cantilever array. This technique is employed in the optically-based commercial array platforms, but sequential switching, on and off, of each laser source is necessary to avoid overlap of the reflected beams on the photodetector. This problem can be elegantly solved using a scanning laser source, where the laser beam is scanned along the array in order to illuminate the free ends of each microcantilever sequentially [23].

Lechuga et al. introduced a new type of optical waveguide cantilever [24,25] that does not need an array of laser sources as the cantilever itself acts as the waveguide in conducting the light. At the exit of the optical cantilever, light can be collected by another waveguide or by a photodetector. This new device has been shown to have a good performance, and it offers an interesting approach to further integration in lab-on-a-chip microsystems.

3.2. Piezo-resistive read-out
Piezo-resistive read-out is based on the changes observed in the resistivity of the material of the cantilever as a consequence of a surface-stress change [26–30]. A piezo-resistive sensor measures the variation in film resistance with respect to surface stress caused by the specific

Figure 2. (A) Scheme of the optical read-out method for a cantilever bending evaluation. (B) Scheme of the piezo-resistive read-out and the Wheatstone bridge configuration.
binding of molecules. For piezo-resistivity to be observable, the electrical conductivity along the thickness of the cantilever has to be asymmetric, which is often accomplished by differential doping of the material. Fabrication of piezo-resistive silicon cantilevers is achieved by asymmetrically coating both sides of them with silicon nitride in such a way that the neutral axis of the cantilever is inside the coating. To measure the change in the resistance, silicon cantilevers must be included in a dc-biased Wheatstone bridge (see Fig. 2B). This configuration is very suitable for further integration into arrays of cantilevers [31].

Piezo-resistive cantilevers, as compared to the optical read-out type, show several advantages:

- piezo-resistive detection can work in non-transparent solutions;
- time-consuming laser alignment is not needed;
- read-out electronics can be integrated on the same silicon chip;
- temperature control can be easily implemented; and,
- they are compatible with miniaturization and array fabrication.

The main disadvantage is the intrinsic noise level that directly affects the resolution and the sensitivity when compared to optically detected cantilevers [32], although reducing the thickness of piezo-resistive cantilevers could increase sensitivity. However, the cross-sectional structure of a piezo-resistive cantilever is complex, with the technological limitations consequent on fabricating thin, highly-sensitive cantilevers. Moreover, piezoelectric read-out requires electrical connections to the cantilever and their isolation from the solution. For all these reasons, the optical method is employed more, and achieves the best LODs [4,10].

4. Fabrication of microcantilever sensors

Silicon, silicon nitride and silicon oxide cantilevers are available commercially with different shapes and sizes, analogous to AFM cantilevers, with typical lengths of 10–500 μm, and ultra-thin cantilevers up to 12 nm thick. However, for specific applications (e.g., highly sensitive biosensors), cantilevers must be designed and fabricated to satisfy their requirements.

Cantilevers are batch fabricated using well-established thin-film-processing technologies that provide low cost, high yield, and good reproducibility. Such fabrication techniques generally include thin-layer deposition, photolithographic patterning and etching, and surface and bulk micromachining. Normally, a sacrificial layer is first deposited on a pre-patterned substrate before depositing the structural material of the cantilever. This structural layer must be free of stress gradient; otherwise, there will be problems with initial bending of the cantilevers. The thickness of the layer must be uniform enough around the wafer to make sure that all the beams will be identical. It is possible to fabricate arrays of thousands of identical cantilevers on one wafer. Fig. 3 shows some fabricated cantilever arrays.

Cantilever sensitivity depends critically on the spring constant: the lower the constant, the higher the sensitivity for measurements in liquids based on the static method. A key factor that dramatically affects the spring constant of a cantilever is the Young’s modulus, which is directly related to the properties of the cantilever material. Cantilevers are normally made of silicon or related materials that have a high Young’s modulus. A cantilever made of a softer material would be more sensitive for static deflection measurements, so polymers with a much lower Young’s modulus than silicon have been used as a substitute material for fabricating cantilevers [33–35]. Among polymers, SU-8 has been shown to be very sensitive, exhibiting a Young’s modulus about 40 times lower than that of silicon. In addition, cantilever fabrication is almost inexpensive, fast and reliable. It also provides a convenient way to realize arrays of multiple sensors and to integrate them into a miniaturized biochemical analysis system [33]. But there is still no proof of biosensing using the polymer for cantilever sensors, mainly due to the difficulty of achieving stable immobilization of the receptor layer.

An easier fabrication strategy is simply coating the silicon cantilever with an analyte-permeable layer, such as a colloidal material [36] or a polymer [37]. Deflection of these cantilevers takes place as a result of analyte-induced...
swelling of the coating, similar to a sponge. Furthermore, it offers an approach to increasing the number of binding sites per cantilever without compromising accessibility by the analyte.

Modifications of cantilever shape and dimensions could also improve the cantilever spring constant; longer and thinner [38,39] cantilevers can have very small spring constants. Microfabrication technologies allow reproducible, inexpensive fabrication of µm-sized cantilevers with high length to thickness ratios. However, thermal motion of the cantilever severely limits the extent to which the spring constant of the cantilever can be reduced [8].

5. Biofunctionalization of the microcantilever surface

Immobilization of bioreceptors on the sensor surface strongly affects the quality of microcantilever analyses, since it influences not only the efficiency of protein and DNA attachment, but also the degree of non-specific binding and the accessibility to its targets. Moreover, the bioreceptor layer directly affects the reproducibility, the selectivity and the resolution of the device. The immobilization process should:

- avoid any change in the mechanical properties of the cantilever;
- be uniform, in order to generate a surface stress as large as possible; and,
- allow accessibility by the target molecule.

Usually, the cantilever sensor has one surface coated with a thin layer of gold (20–100 nm), which provides an excellent chance of using self-assembled monolayer (SAM) thiol chemistry (see Fig. 4). Immobilization of both DNA and proteins on gold surfaces using thiol-SAMs is a well-known chemistry and has been widely used in many other biosensing applications, such as surface plasmon resonance (SPR) [40], quartz crystal microbalance (QCMB) [41], or electrochemical (EC) measurements [42].

5.1. DNA immobilization

Direct coupling of DNA probes by self-assembly of thiol-labeled oligonucleotides is a common, easy use for gold-coated microcantilevers. Herne and Tarlov characterized the immobilization of single-strand DNA oligonucleotides on gold via sulfur linkage [43]. The sulfur atom causes a great change in surface stress during DNA immobilization in contrast to non-modified DNA, as shown in Fig. 5. Most DNA-biosensing applications performed with microcantilever technology are based on this strategy, but, to date, there is no report of hybridization experiments on cantilevers, considering, for example, the effect of sulfur-linker form (thiols vs sulfides or disulfides).

Surface coverage directly affects the hybridization rate [43]; furthermore, the buffer concentration of the thiolated DNA solution has a great effect on surface coverage, reducing the non-covalent interactions at low salt concentrations. Herne and co-workers also demonstrated that a mixed layer with mercaptoglycol (MCH) enhanced the hybridization rate and minimized the physical adsorption of DNA probes on gold surfaces by removing the weaker adsorption contacts between the nucleotide chain and the gold. In this way, the majority of the immobilized DNA probes are accessible for hybridization with the complementary strand.

5.2. Protein immobilization

The covalent adsorption of proteins on gold surfaces of cantilevers can be achieved by a wide variety of chemical procedures, ensuring the reproducibility and the stability of the protein coating. The main disadvantage of chemical adsorption is that some of the functional groups could randomize the orientation of the active sites of the protein, preventing binding with the analyte. Hence, key is the choice of the appropriate immobilization procedure in order to avoid a low rate of activity of the protein receptor.

There are several strategies that could be employed. A very interesting one is covalently immobilization of carboxylate-terminated alkanethiols (e.g., 11-mercaptoundecanoic acid) followed by esterification of the carboxylic

![Figure 4](http://www.elsevier.com/locate/trac)
groups with 1-ethyl-3-(3-dimethylaminopropyl)-carbo-
diimide (EDC) and N-hydroxysuccinimide (NHS) [44]. EDC is a water-soluble derivative of carbodiimide that catalyzes the formation of amide bonds between carboxylic acids or phosphates and amines by activating carboxyl or phosphate to form an O-urea derivative. This derivative reacts readily with nucleophiles. NHS is often used to assist carbodiimide coupling in the presence of EDC. The reaction includes formation of the intermediate active ester (the product of condensation of the carboxylic group and NHS) that further reacts with the amine function of proteins to yield the amide bond and the final immobilization of the protein on the gold-coated cantilever.

Another option is to use cystamine modified with glutaraldehyde and the subsequent attachment of the protein through an amine group [12,45]. In addition, we can immobilize biotinylated proteins on avidin monolayers that can be achieved with EDC or cystamine chemistry.

Finally, the immobilization procedure developed by Park and Kim employs the reaction of sulfosuccinimidyl 6-[3-(2-pyridyldithio)pro-pionamido] hexanoate (sulfo-LC-SPDP) with the protein NH2 groups to give amide linkages; addition of dithiothreitol (DTT) reduces the disulfide to give a thiol, which then self-assembles on gold [46].

5.3. Deposition onto array systems
The immobilization of different bioreceptors on each cantilever of an array is a complicated task. There are several commercial platforms devoted to the specific functionalization of individual cantilevers. The different strategies range from the Autodrop platform (Microdrop, http://www.microdrop.de/index.html) and CantiSpot platform (Cantion, http://www.cantion.com/content/

Figure 5. (A) Deflection signals of a single cantilever sensor for 2 μM ss-DNA (27 mer) modified at the end with a thiol group and the corresponding unmodified ss-DNA. It is shown that no deflection signal is observed for the unmodified ss-DNA case. (B) Using the array platform showed in Fig. 3, we have measured simultaneously the immobilization of a 2 μM thiolated DNA (27 mer) on each cantilever.
products/CantiSpot), based on a spotter that delivers pL droplets, to capillary-based systems, such as Cantisens FU-401 (Concentris, http://www.dkshins.co.kr/pda_data/Concentris/Cantisens%20FU-401.pdf).

AutoDrop moves up to eight dispensers or pipettes in an area of $200 \times 200 \times 80$ mm, automatically filling, cleaning and dispensing. Bietzsch et al. [47] described the uniformity of the deposition of thiolated DNA and others alkenothiols using the microdrop system.

The CantiSpot has a spotter capable of delivering 100-pL droplets. The spotter is connected to a computer-controlled syringe pump for controlled aspiration and dispensing of reagents.

The Cantisens FU-401 platform offers a way of immobilizing biomolecules on cantilever arrays using capillary techniques that allow up to four cantilevers to be functionalized simultaneously.

A completely different approach, aiming to use a microfluidics system with independent flow path for each cantilever in an array, is under development in several laboratories [48].

6. Analytical applications

6.1. Chemical sensing

Nanomechanical sensors using gold-coated cantilevers were first used for detecting volatile compounds. Molecules, such as mercury or mercaptoethanol, displayed an intrinsic affinity for gold that allowed their attachment to the cantilever surface [49,50]. However, cantilever coatings, such as gold, make it difficult to expand detection to other compounds. Selectivity was enhanced by using organic coatings, such as polymers. Several coatings, such as poly-N-vinyl pyrroldinone (PVP) and poly-ethylene glycol (PEG) have hydrophilic properties and are very suitable as coating materials to measure changes in relative humidity [51].

Commercially-available polymers have also been used to coat cantilevers for differentiating between different volatile organic compounds (VOCs) in air. Baller et al. developed a Nanotechnology Olfactory SEnsor (NOSE) to characterize and to identify gaseous analytes [13].

In the field of explosives, Pinnaduwage et al. reported measurement of trinitrotoluene (TNT) in a small, localized explosion on an uncoated piezo-resistive microcantilever [15,52].

Other functionalization schemes, such as those based on self-assembly, have been tried [53]. Ambient liquids allowed the detection of several compounds, such as ions and, especially, biological compounds that could not be detected in air. First, experiments on solution were focused on ion detection and measurement of changes in surface stress induced by changes in pH or ion concentrations. It was found that the cantilever response depended upon both pH and the ionic strength of the aqueous medium [54].

Functionalization of cantilevers with self-assembled monolayers, such as alkythiols ended in different chemical groups, has also been used. The pH responses were in the range 15–50 nm/pH and depended on cantilever type, surface treatment and pH range [55,56].

Heavy-metal ions and ions in general have also been studied. Ji et al. used thiol-derivatized calixarene and crown-ether macrocycle-functionalyzed cantilevers to detect Cs$^{+}$ ions in the range $10^{-11}$–$10^{-7}$ M and K$^{+}$ in the $10^{-4}$ M range [57].

Other functionalization schemes have shown that cantilevers were able to detect, with great accuracy and selectivity, different ions, such as CrO$_{4}^{-2}$ [58], Cu$^{2+}$ or Pb$^{2+}$[59].

Table 1 compares the performances using the different strategies described above.

6.2. Biosensing applications

An excellent example in the field of genomics was the detection of a single-base mismatch with an LOD of 10 nM reported by Fritz et al. [6]. For such detection, they used an array of two cantilevers with a control (non-complementary) oligonucleotide in one of them and the DNA probe (complementary) immobilized in the other, giving hybridization-deflection signals as small as 10 nm and 16 nm for 12-mer and 16-mer DNA targets, respectively, and displaying a deflection noise of 0.5 nm.

McKendry et al. detected 75 nM of target oligonucleotide using an array of eight microcantilevers [4] (see Fig. 6).

Both the above results included specific immobilization by microcapillary with a 40 µM solution of the thiolated DNA probe.

Thundat et al. reported the discrimination of single-nucleotide polymorphism with a single cantilever [9]. This clearly contrasted with the data from Alvarez et al., who found it impossible to detect hybridization without a reference cantilever [19].

Other DNA detection schemes have been reported, as for example the one which used a capture oligonucleotide combined with a DNA probe attached to a gold nanoparticle. This method can detect at least 0.05 nM and is able to discriminate a single mismatch measured by resonance [60]. An interesting paper by Wu et al. described the effect of different phosphate buffer concentrations, suggesting that electrostatic repulsive forces between neighboring DNA molecules must play a role in cantilever motion and demonstrating that configurational entropy changes and intermolecular interactions can control the direction of motion in nanomechanical sensors [21].

The possibility of obtaining protein microarrays with lower cost fabrication methods, no labeling of the target protein and improved sensitivity has triggered the
development of protein biosensors based on arrays of cantilevers. Numerous studies have been reported using antibodies as receptors for detecting proteins. Wu et al. [10] reported the detection of two isoforms of prostate-specific antigen (PSA), a useful marker for earlier detection of prostate cancer, with an excellent range of discrimination and an LOD of 6 ng/ml (deflection signal 20 nm) in a background of 1 mg/ml of BSA protein.

Wee et al. [61] have also reported the detection of PSA and C-reactive proteins (CRP), which is a specific marker of cardiac disease, by means of an electromechanical biosensor using self-sensing piezo-resistive micro-cantilevers.

In addition, a novel development for early osteosarcoma detection has been described, sensing the interactions between vimentin antibodies and antigens with a single cantilever-based biosensor [62] and supporting the idea that cantilever biosensors can provide a suitable platform for life-sciences research.

Other clinical applications have included the detection of different pathogens, such as *Salmonella enterica* by Weeks et al. [14], *Vaccinia virus* by Gunter et al. [63] and fungal spores from *Aspergillus niger* by Nugaeva et al. [64].

Monitoring concentrations of specific pesticides plays an essential role in the environmental control field. An example of the application of a cantilever-based biosensor in this area was reported by Alvarez et al. [12] for the detection of the organochlorine insecticide compound dichlorodiphenyltrichloroethane (DDT). A synthetic hapten of the pesticide, conjugated with bovine serum albumin (BSA), was covalently immobilized on the gold-coated side of the cantilever; specific detection was then achieved by exposing the cantilever to a solution containing the specific monoclonal antibody to the DDT-hapten derivative. The specific binding of the antibodies on the sensitized side of the cantilever was measured with nmol sensitivity. Finally, a competitive assay was performed, with the cantilever exposed to a mixed solution of the monoclonal antibody and DDT, and direct detection was achieved. With this detection strategy, DTT concentrations as low as 10 nM were detected, involving deflection signals in the 50-nm range (see Fig. 7). Many other applications have been described for detection of pesticides and avidin-streptavidin [7].

### Table 1. Analytical comparison of different chemical sensing applications

<table>
<thead>
<tr>
<th>Compound</th>
<th>Detection limits</th>
<th>Technique</th>
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<tr>
<td>Mercury</td>
<td>10^{-11} M</td>
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<td>[49]</td>
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<tr>
<td>2,4-dinitrotoluene (DNT)</td>
<td>300 ppt</td>
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<td>Trinitrotoluene (TNT)</td>
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<td>Ca^{2+} / K^{+}</td>
<td>10^{-11} / 10^{-4} M</td>
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**DNA hybridization:**
- Mismatch
  - 10 nM
  - Optical read-out
  - [6]
- 8 cantilevers array
  - 75 nM
  - Optical read-out
  - [4]
- Nanoparticle labeling
  - 0.05 nM
  - Piezo-resistive
  - [60]

**Proteins and pathogens:**
- Two isoforms (PSA)
  - 6 mg/ml (20 nm signal)
  - Optical read-out
  - [10]
- C-reactive proteins
  - 10 mg/ml
  - Piezo-resistive
  - [61]
- *Salmonella enterica*
  - 25 bacteria
  - Piezo-resistive
  - [14]
- *Vaccinia virus*
  - 20 mg/ml
  - Piezo-resistive
  - [63]

**Environmental control field:**
- DDT
  - 10 nM
  - Optical read-out
  - [12]
The efficacy of aptamers as the bioreceptor element has been widely probed for a number of biosensing platforms [65–67]. By using aptamers as bioreceptors, Savran et al. developed novel, label-free, protein detection using cantilever biosensors. The sensor used two adjacent cantilevers that constituted a sensor-reference pair and allowed direct detection of the differential bending between them. One cantilever was functionalized with aptamers selected for Taq DNA polymerase, while the other was blocked with single-stranded DNA. The binding of the polymerase-aptamer induced a change in surface stress, which caused a differential cantilever bending in the range 3–32 nm, depending on the ligand concentration [11].

7. Commercial cantilever biosensor platforms

There are available several commercial platforms based on cantilever-array sensors, and Table 2 shows a list of them, including the patents and technologies employed.

Concentris offers micromechanical, silicon, cantilever arrays for static and dynamic measurements. Static cantilever arrays are suitable for applications where extremely small forces must be detected, as in biochemical sensing. Static cantilever arrays CLA-500-010-08 and CLA-750-010-08 are suitable for chemical and biochemical sensing or nanocalorimetry, with eight P-doped Si levers per array, a thickness of 1 μm and lengths of 500 μm and 750 μm, respectively.

Dynamic cantilever arrays are designed for detecting very small mass changes on the cantilever surface. Dynamic cantilever arrays CLA500-070-08V and CLA500-070-04V2 with a thickness of 7 μm and resonant-frequency steps between cantilevers of 40 kHz are suitable for detecting small changes in mass load resulting from adsorption or desorption of molecules.

The Cantion product portfolio comprises the Canti Chip 4, an array of four nanomechanical cantilevers with integrated electrical read-out and the Canti Lab 4 read-out instrument with control of the fluidic system.

Table 2. Commercialized cantilever array sensors (May 2005). References for the patents can be found at the corresponding product website

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<td>Scentris cantilever sensor</td>
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<td>San Francisco, USA</td>
<td>Kalinex Inc. [<a href="http://www.kalinex.com">www.kalinex.com</a>]</td>
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Figure 7. DDT pesticide detection using a single microcantilever sensor by real-time competitive immunoassay (see related text for details). The cantilever surface was regenerated with 100 mM HCl to break the hapten/antibody complex.
This system has been designed as an open platform that allows the user to functionalize each cantilever individually using the Canti Spot platform and has been optimized for both gas and liquid operation.

The VeriScan 3000 System from Protiveris is a multiplexed, label-free biosensor platform designed to measure molecular interactions, such as those between proteins, antibodies, antigens, or DNA. The system is capable of measuring 64 microcantilever sensors in real time and incorporates patented laser technologies, advanced electronics, microfluidics, a proprietary biochip, and advanced software capabilities.

Recently, the start-up company Kalinex Inc (San Francisco, CA, USA) commercialized a biochemo-optomechanical microcantilever-array chip (BioCOM) developed at the University of California, with 1000 microcantilevers on a chip. It can test 100–200 different compounds, with the remainder of the cantilevers redundant. However, the BioCOM chip is not yet ready for the market.

Other companies [68] are developing fully integrated microsystems platforms including cantilever sensors, optical excitation and detection systems, microfluidics and electronics chips in a single package, which is intended to be used as a hand-held device for decentralized analytical testing.

8. Conclusions and future goals

In this review, we have provided an overview of most technical aspects of the new nanomechanical biosensors, the surface chemistry for receptor immobilization and the state of the art of the applications of these devices. The microcantilever-based biosensor is a promising technology that is emerging as a suitable solution for important problems. Improvements in reproducibility and sensitivity, and integration of microfluidics and detection systems are the main aims of current research.

Future trends in nanomechanical biosensors will mainly address new fabrication methods to enhance cantilever response. One is to work on cantilever miniaturization to the nanoscale. Nanocantilevers display large spring constants and are unsuitable for detecting adsorption-induced cantilever bending, but they can be employed for highly sensitive, dynamic measurements. Nanocantilevers are expected to provide ultra-high-sensitivity mass detection, ultimately approaching the single-molecule level [8].

Approaches to fabrication are also focused on nanostructures to increase the surface area. Gold-nanoparticle coating and sub-μm surface channels are some examples of this trend [69,70]. Such an approach dramatically decreases the spring constant, and can increase cantilever response by two orders of magnitude, at least [70]. Furthermore, nanostructured surfaces can substantially increase the number of binding sites without compromising accessibility by the analyte.

Future developments also include large cantilever-array fabrication along with integrating the optical or piezo-resistive read-out and the microfluidics, with the final objective of developing a portable biosensor microsystem for high-throughput screening.

To sum up, microcantilever-based biosensors comprise a continually growing novel technology and, because of their great capabilities, offer an alternative to current biosensor technologies. Cantilevers will play an essential role in the immediate future of nanobiotechnology.

Acknowledgement

The authors would like to acknowledge financial support from the European Project Optonanogen (EU Contract IST-2001-37239) and from the Spanish national projects TIC2002-10473-E and GEN2001-4856-C13-11.

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