Microcantilever-based platforms as biosensing tools

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Received 28th April 2009, Accepted 10th December 2009
First published as an Advance Article on the web 13th January 2010
DOI: 10.1039/b908503n

The fast and progressive growth of the biotechnology and pharmaceutical fields forces the development of new and powerful sensing techniques for process optimization and detection of biomolecules at very low concentrations. During the last years, the simplest MEMS structures, *i.e.* microcantilevers, have become an emerging and promising technology for biosensing applications, due to their small size, fast response, high sensitivity and their compatible integration into "lab-on-a-chip" devices. This article provides an overview of some of the most interesting bio-detections carried out during the last 2–3 years with the microcantilever-based platforms, which highlight the continuous expansion of this kind of sensor in the medical diagnosis field, reaching limits of detection at the single molecule level.

Introduction

The final aim of any biosensor technology is the development of a fully integrated, cheap, portable and reliable single platform, able to detect and identify simultaneously different molecules in real time with high sensitivity, even at the single cell and single molecule level. Biosensors based on microcantilevers offers many of the properties required for that goal, such as a tiny sensor area, a label-less detection method, low-cost fabrication and mass production, and compatibility with CMOS (complementary metal-oxide semiconductor) technology that can be easily scaled up; currently it is possible to fabricate arrays of tens to thousands of microcantilevers. The working principle of nanomechanical transducers, and specifically of cantilevers, involves the translation of the biochemical reaction occurring on top of the cantilever surface into a mechanical motion. Any change produced on the sensing layer as a consequence of an external stimulus will cause a response of the microcantilever. The applications performed with this type of sensors have experienced a spectacular rise in the last few years including the detection of gases, chemical and biological compounds. But due to the high potential impact in diagnosis, the biosensing area is the one which has received the strongest effort, covering fields that range from genomic and proteomic, to environmental, industrial control, clinical diagnosis or drug screening and pathogens detection. A recent publication by Ríos *et al.* presented an excellent collection of the applications of the microelectromechanical sensors (MEMS) published in the analytical field including the detection method employed in each case.[1]

The limits of detection achieved with these sensors are comparable or even better than the ones achieved with other non-labeled techniques commonly used as reference systems, such as surface plasmon resonance (SPR). However, even if these

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devices are currently commercialized by few companies (*i.e.* Concentris GmbH), biosensors based on microcantilevers still show limitations that need to be solved before they can be turned into a robust commercial tool, comparable to other techniques such as SPR or AFM that are commercialized for different companies worldwide (such as Biacore, Sensia S.L., Veeco Instruments Inc., Nanotec S.L., …). Most of the existing challenges in microcantilevers biosensing are related with the necessity of working in liquid environments, which force more complex technological developments and strategies as compared to working in the gas phase. The complex relationship between the binding event and the cantilever response, the effect of the surrounding media on the binding detection (pH or ionic strength changes, damping,) or the sensing layer formation over the cantilever surface (reproducibility, packaging, cleanness,…) are some of the current constraints. Many different alternatives and promising devices has been proposed in the literature to overcome some of these questions and to increase the sensitivity, either focused on the chemistry, on the detection method or in the MEMS size and shape. For example, the development of high frequency resonators has become one of the most promising options,[2–4] especially for mass detection. The integration of carbon nanotubes (CNTs) with the MEMS has also been considered for biomolecular sensors due to the size, properties and small mass of the CNTs.[5]

In this review, we pointed our attention on the biosensing applications (in liquid phase) carried out with microcantilevers during the last few years. We have analyzed the performance reached by the microcantilever-based sensors and which are the current limitations from a biosensing viewpoint, studying the trends and benefit of the mechanical biosensors in comparison with other non-labeled biosensing techniques.

Modes of operation

The adsorption of molecules and the biomolecular recognition over a cantilever surface may lead to changes in the cantilever bending or shifts in its resonance frequency, as is shown in Fig. 1. The detection of these responses is usually referred as static and dynamic modes of operation, respectively.

Working in the *static* mode, the bending arises as a consequence of a surface stress change induced by any molecular reaction which takes places on only one of the cantilever surfaces. The induced surface stress change could be positive or negative, depending on the surface deformation generated. The factors and phenomena responsible for this change is today not fully understood due to the complex equilibrium between the sensing layer, and the surrounding water molecules, the bulk solution, the target molecules, ions, *etc.* Interactions coming from electrostatic, hydration, steric and van der Waals forces, changes in the surface hydrophobicity or conformational changes of the adsorbed molecules play an important role in the final bending.[6,7] The easiest and most extended model to study the surface stress produced on cantilevers is based on the work of G.G Stoney in 1909. Looking for higher accuracy, some works have presented modifications on this model, depending on the thickness or roughness of the surface.[8] Other energy-based models studied the dependence of the cantilever bending with the density of the adsorbed atoms/molecules and the properties of the substrate.[9]

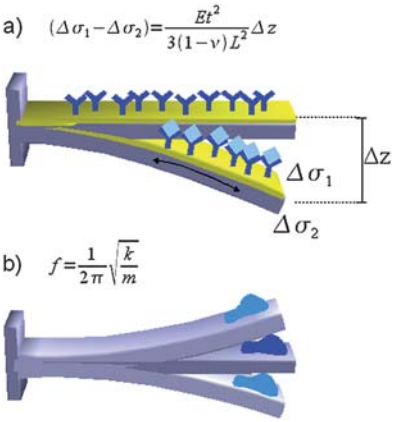

Fig. 1 Schematic representation of the microcantilever-based biosensor working principles, a) static mode and, b) dynamic mode.

Stoney's model relates the total surface stress change between the top and the bottom sides ($\Delta\sigma_1 - \Delta\sigma_2$) with the cantilever free end displacement, Δz, the Young's modulus, E, the Poisson coefficient, ν, and the cantilever length, L, and thickness, t, by,

$$(\Delta\sigma_1-\Delta\sigma_2) = \frac{Et^2}{3(1-\nu)L^2}\Delta z$$

For sensing biomolecular interactions in the static mode, only one surface of the microcantilever must be previously biofunctionalized and this can be a complex task especially when arrays of microcantilevers are employed.

In contrast to the static case, the *dynamic* mode does not require the functionalization of only one cantilever surface, as the cantilever resonance frequency change depends on the total mass adsorbed on both sides. In this mode, the microcantilever is used as a microbalance and extremely high sensitivities can be obtained (in the attogram regime), overcoming other similar and well-known mass detectors, such as the quartz crystal microbalance (QCM).[10] In first approximation cantilevers behave like a harmonic oscillator, and the mass change on a rectangular cantilever will produce a reduction on the resonance frequency, which can be estimated from:

$$\Delta m = \frac{k}{0.96\pi^2}\left(\frac{1}{f_0^2}-\frac{1}{f_1^2}\right)$$

where f_0 and f_1 are the fundamental resonance frequency before and after the mass adding, respectively.

Recent studies have demonstrated that surface stress can induce a microcantilever stiffness change, due to strain-dependent surface stress (elasticity), which can cancel or make negligible the resonance frequency change due to the added mass.[11–13] The induced cantilever stiffness change can produce a resonance frequency shift as high as the added mass, but increasing the microcantilever resonance frequency, depending on the attached protein density and thickness and the cantilever length.[14] Actually, this is one of the questions to be solved in order to avoid errors in the characterization of biological agents, being necessary to identify and detach the frequency shifts coming from the added mass and the stiffness changes.[15]

When working in the dynamic mode, the resolution of the system, Δf, is determined by the quality factor, Q, following the

expression $\Delta f = f/Q$. The quality factor quantifies the energy dissipation and is defined as the ratio between the mechanical energy accumulated and dissipated per vibration cycle. Under liquids environments, the quality factor shifts toward much lower values than in air, due to the damping effect of the viscous surroundings, which decrease abruptly the overall sensitivity. For that reason, this way of operation is more difficult to implement and, until recently, most of the cantilever biosensors were based on the static mode.

Methods of detection

A sensitive readout system is crucial for monitoring the nanomechanical motion induced on the cantilever. Among the most extended readout schemes for biosensing are the optical, the piezoresistive and the piezoelectric ones.

The optical method is simple to implement and shows a linear response with sub-angstrom resolution, and is currently the most sensitive method for measuring nanomechanical motions. In the optical lever scheme, the cantilever free end movement is detected by measuring the reflected laser beam displacement into a position-sensitive photodetector (PSD). The optical detection mechanism present some disadvantages related with changes in the optical properties of the environment and its bandwidth. Its implementation in arrays of cantilevers is technologically challenging, as it requires an array of laser sources with the same number of elements as the cantilever array. The beam displacement could be measured by using an array of photodetectors,[16] adding alignment complications, or using just one photodetector and sequentially switching on and off each laser source.[17] A different alternative to overcome the array implementation problem is to use a CCD camera and an array of microcantilevers with paddles at its end for increasing the light reflectivity.[18]

The piezoelectric and piezoresistive detection methods are based on the integration of a piezoresistive or piezoelectric material, respectively, on the microcantilever. Both methods allow a higher and simpler integration when working with arrays of microcantilevers, but they have lower sensitivity than the optical method. The possibility of self-exciting/sensing of piezoelectric cantilevers makes them very interesting for dynamic detection, because of the higher Q that can be achieved.

Other techniques that have also solved the problems for the integration of the detection readout system in arrays platforms are the fabrication of silicon microcantilevers with an embedded MOSFET at the clamping area,[19] and the optical waveguide microcantilevers (OWC), where the principle of operation is based on the dependence of coupling efficiency between two butt-coupled waveguides on their misalignment with respect to each other.[20,21] A very interesting alternative for the coupling of the light into the optical microcantilever is the fabrication of embedded diffraction gratings,[22] simplifying even more the optical readout sub-system.

Cantilever sensitivity

The sensitivity of the cantilever response will depend on its mechanical properties, which are determined mainly by their spring constant and resonance frequency. Both parameters depend on the cantilever material and its geometry. The spring constant, k, and resonance frequency, f_0, for a rectangular cantilever clamped at one end are given by,

$$k = \frac{Ewt^3}{4L^3}, \quad \text{and} \quad f = \frac{1}{2\pi}\sqrt{\frac{k}{m}}$$

where E is the Young's modulus, m is the cantilever effective mass, w is the width, t is the thickness, and L is the length, respectively.

The cantilever dimensions and the material chosen will depend on the working detection method (static or dynamic), the bio-application requested and the available fabrication technology. Cantilevers with smaller spring constants (high aspect ratio) give cantilevers more sensitive to surface stress changes, while shorter and thicker cantilevers, with higher resonances frequencies, are more sensitive for dynamic detection.

In general, microcantilevers can be fabricated of any shape and from substantially any material utilized in the microelectronics industry, *i.e.* crystalline or poly-silicon, silicon nitride, silicon dioxide, or other materials such as polymers or piezoelectrics. Nowadays, the most common material for cantilever fabrication is still the single crystalline silicon, as for the ones shown in Fig. 2a, due to its low internal stress and its well established and reliable fabrication process. Different beam-based structures have been fabricated by using this technology, such as T-shaped microcantilevers, for reducing the initial dispersion bending in arrays of microcantilevers or sensor/reference cantilevers supported by L-shaped thick structures that connect them to the die, for interferometric measurements.[23,24] Other materials like SU-8 polymer, have a lower Young's modulus than silicon and can have a good sensitivity for static deflection measurements even if they do not have a high length : thickness ratio ($t \sim 1\mu m$), however the chemistry required for its functionalization is more complex than when working with silicon. Due to, in general, its lower

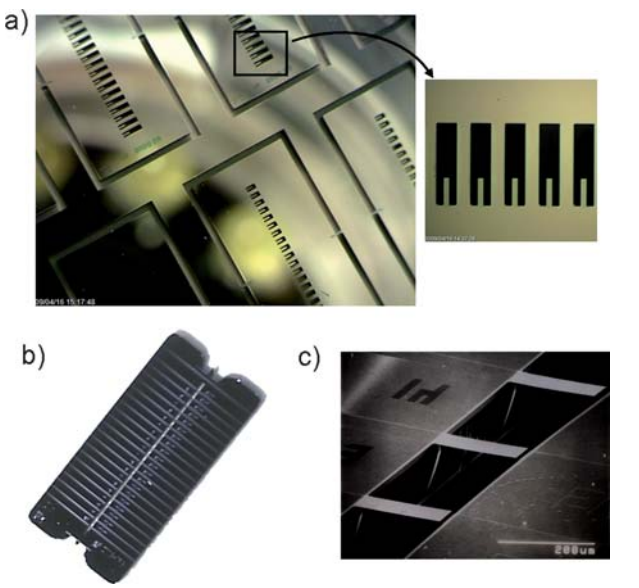

Fig. 2 a) Photograph of the Si wafer with the fabricated chips of arrays of 20 microcantilevers; each cantilever is inside an individual window (that can have different sizes). Cantilevers dimensions are 200 μm length, 20 μm widths and 0.335 μm thick. b) Photograph of the fabricated OWC chip and c) SEM image of the waveguide microcantilevers.

Young's modulus, polymeric materials are not suitable for dynamic detection where the trend is towards stiffer and smaller cantilevers to increase its sensitivity. Piezoelectric microcantilevers are usually thicker because of the fabrication process (with higher resonance frequencies) and are more suitable for dynamic applications. For piezoresistive microcantilevers, high aspect ratio cantilevers are optimal for point-loading applications while low aspect ratio cantilevers are better for surface stress-loading sensors applications.[25]

In the case of the optical waveguide microcantilever there are different factors that can affect the system sensitivity, like the distance from the cantilever edge to the collecting waveguide, the coupling efficiency or the cantilever material and rigidity. Apart from the initially proposed optical silicon waveguide microcantilevers,[20] shown in Fig. 2b, new approaches has been presented recently using different materials, such as indium phosphide or polymers.[26–28] Other work proposes the use of silicon photonic crystals for guiding the light through the microcantilever.[29] And Li *et al.* demonstrated the integration of a pair of end-to-end coupled waveguide cantilevers and a grating coupler at the end of the waveguides to avoid diffraction limits.[2] However, to our knowledge, no biological applications have been reported till now with any of these configurations.

Besides the cantilever shape and material, the morphology and cleanliness of the sensing surface can have a dramatic effect on the final surface stress generated. A thin gold layer is usually deposited over one cantilever side to increase the laser reflectivity and facilitate the functionalization, influencing the final microcantilever bending. A correlation between surface stress and roughness of the gold surface has been reported.[30,31,8] Tabar-Cossa *et al.* observe a 25-fold amplification of the change in the surface stress by increasing the average gold grain size of the sensing surface from 90 to 500 nm, and a reduction on the surface stress response and a different profile shape for unclean surfaces.[31] Therefore, the surface morphology and the cleaning process must be well controlled because the reproducibility of the cantilever functionalization and the detection signal are directly correlated to that.

Biosensing applications

Bimaterial microcantilevers

As in any other biosensor, a reactive and specific biolayer in contact with the transducer is needed for performing any detection of chemical or biological molecules. To preserve the reactive layer functionality (*i.e.* structure and active groups), the recognition process must be performed under biocompatible solutions. The reactive layer provides the selectivity and specificity to the biosensor, due to the high capacity of the biological molecules to recognize a specific analyte. As we already mentioned, the standard micro/nanomechanical transducers are made out of semiconductors, metal oxides and inorganic materials in general, while the range of reactive layers, and therefore of applications, is enormous, due to the high variety of biomolecules to be detected.

Typical reactive layers widely used for these combined biosensors structures are self-assembled monolayers (SAMs) and numerous classes of polymers, hydrogels or brush macromolecules[32] (see Fig. 3). The SAMs is a widely employed

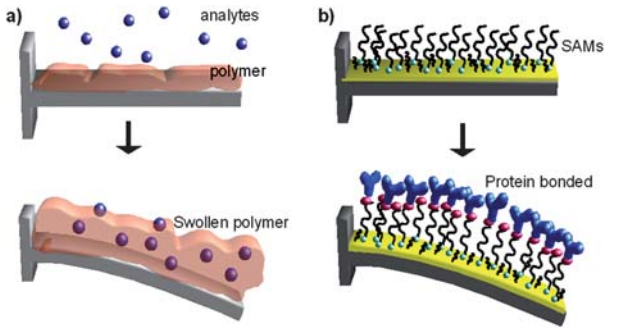

Fig. 3 Scheme of the response of microcantilevers when using a) a polymer or b) SAMs sensing layers.

technique in many different fields, which has been intensively investigated because it renders the formation of robust and compact monolayers in an easy and controllable way. This method allows controlling the surface functionality depending on the functional group of the organic chains, either working with silanes or thiols, opening a wide range of applications, from covalent protein binding to pesticides, ions or plastic explosives detection. The physics behind the SAM response and the cantilever movement has been extensively studied and is still under discussion. Recently, Norman *et al.* have studied the electrochemical actuation of microcantilevers modified with a self-assembled ferrocenylundecanethiolate monolayer, finding that the cantilever responds to collective in-plane reorientational motions (*i.e.* monolayer volume expansion) rather than reporting individual biochemical events.[33] The oxidation and subsequent reduction of the organic monolayer induce a reversible surface stress on the cantilever, and its corresponding motion, due to the lateral pressure exerted by an ensemble of reorienting ferrocenium-bearing alkylthiolated upon each other. A common configuration for the covalent binding of proteins is the amino- or carboxyl-ended SAMs and a cross-linker, such as glutaraldheide or 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) with *N*-hydroxysuccinimide (NHS). With these configurations it has been reported more than 100 regenerations of the surface,[34] by removing the target molecule after binding, which support the robustness of the method and the reusability of the system.

The application of polymers (from 3D networks to brushes) as an active surface has become a very interesting alternative to the employment of SAMs due to the larger differential stress which is produced. As for SAMs, the sensing application is determined by the polymer layer chemistry selected. The polymer layer covering the cantilever will swell during the analyte absorption, inducing deformations in the cantilever, thus amplifying the cantilever bending, see Fig. 3b. Several coatings, such poly-*N*-vinyl pyrrolidinone (PVP) and poly-ethylene glycol (PEG) have hydrophilic properties and are quite suitable as coating materials to measure changes in relative humidity, or as inertness covering for referenced cantilevers.[35,36] Zhou and co-workers demonstrated the fast and reversible actuation and electroactuation of cantilevers coated with polyelectrolyte brushes, which experience large conformation changes in response to the pH and to the applied voltage, respectively.[37,38] Combinations of microcantilever and polymer layers were used as an artificial nose for the detection of

solvents, perfume essences and beverage flavors by tracking the diffusion process of the molecules into the polymer layers.[39] The final sensitivity and selectivity of the system depends on the thickness of the coating, increasing the bending response when increasing the polymer film thickness. Other works showed the increase in the cantilever thermal sensitivity by using a trilayered (ceramic–metal–polymer) approach, with polymeric layers reinforced with different nanoparticles chemically grafted to the metal-coated surface.[40] New routes, combining a polymer with two specific functional monomers have been proposed.[41] The monomers confer to the polymer the ability to react with nucleophilic species on biomolecules and with glass silanols. This route has been successfully applied for the detection of DNA hybridization and protein/protein interactions.

For cantilever biosensors operating in the surface stress mode, a non-reactive layer/coating on the opposite side is highly recommended to avoid additional stress from specific or unspecific bindings that would cancel the cantilever bending due to the specific signal.

Functionalization of microcantilever arrays

One of the main advantages of nanomechanical-based biosensors is the possibility of working with several cantilevers simultaneously, analyzing different compounds dissolved in the same solution and at different concentrations. The cantilever sensitivity for a specific analyte is given by the reactive layer, which means that the multidetection of analytes is only possible if only one surface of each cantilever is functionalized with a different active layer. For that propose, there are several techniques that allow the "external" or previous functionalization of individual microcantilevers by the localized deposition of tiny drops of solution (few μl) directly over the cantilever surface. A control humidity chamber is needed to maintain the thin layer of solution over the cantilever surface time enough to obtain a well-formed active layer. The drop deposition can be done by inkjet printing or contact printing (by using dip-pen nanolithography).[42,43] Once the layer is formed, the microcantilever is inserted into a fluid cell to carry on the sample detection. There are several commercial available systems for functionalization either using inkjet nozzles (*e.g.* Microdrop system from Microdrop Technologies, Germany) or cantilevers with an integrated microfluidic, (*e.g.* Nano eNabler system from Bioforce Nanosciences, USA). Fig. 4a shows sequential images of drops deposition over the microcantilever surface, performed with a Nano eNabler system, until forming a thin layer covering the entire surface. Arrays of glass microcapillaries where the microcantilevers can be introduced have also been used for this kind of applications,[43] wetting both cantilever sides. Other approaches are the use of microcantilevers with integrated channels, Fig. 4b,[44] or polymeric flow cell with individual channels for each microcantilever,[16] Fig. 4c, which have some advantages such as the detection of the sensing layer formation (*i.e.* real-time checking of the layer condition), giving a faster biofunctionalization and avoiding possible samples contamination.

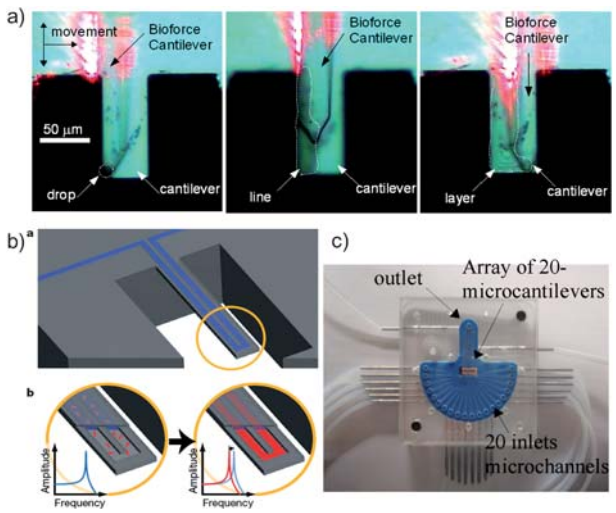

Fig. 4 Methods for the functionalization of array of microcantilevers. a) Contact printing (nano e-Nabler), b) embedded microchannels (Reprinted by permission from Macmillan Publishers Ltd: Nature (ref. 44), copyright 2007) and, c) microfluidics with individual microchannels.

Other commonly used and well controlled techniques like spin coating of polymers, dip coating, or silanes/thiols functionalization by vapor deposition are, in general, not suitable for the selective functionalization of only one cantilever of an array of several microcantilevers.

Besides the detection of multi-analytes with different cantilever/sensing layers, microcantilever arrays platforms allows using cantilevers as reference for performing differential measurements. This will avoid false or anomalous cantilever responses due to refractive index changes, temperature variations or non-specific adsorptions.[45,46] With this purpose, a soft layer biochemically inert to the target under study is generally deposited over neighboring cantilevers and even on the opposite side of the active cantilever.[36,35] A recent research suggests that the reference cantilever coating should not only be chosen according to its chemical inertness but also to the similarity of the elastic properties of the active layer.[47]

Static detection

The static operation principle is the most extended for the detection of chemical and biological compounds in the liquid phase. So far, very low target concentration detection, as well as new ways for quantifying and understanding binding interactions has been already published using the static mode. The first application of nanomechanical platforms for biosensing were performed in 1996, in the proteomic field, with the detection of unspecific adsorption of bovine serum albumin (BSA) on a hydrophobic cantilever surface.[48] After that, Moulin *et al.* measured the surface stress induced by conformational changes of proteins like BSA and IgG adsorbed onto a gold surface.[49] But it was the work published by Fritz *et al.*, in 2000, that was the landmark one for this new biosensing technique.[50] In this work, the deflection of two microcantilevers, initially activated with different DNA chains sequences, were measured simultaneously. The hybridization of complementary oligonucleotides showed that a single base mismatch between two 12-mer oligonucleotides was clearly detectable. This was followed by a large number of publications in the genomic field, for the discrimination of a single DNA mismatch at discrete locations in the DNA chain,[51]

the detection of different DNA sequences in parallel,[52] or the study of the forces playing a role in the surface stress change induced by the hybridization signal.[53] The published results opened the question about the necessity of using cantilevers of reference for those applications,[54,55] which paved the way for the use of arrays of microcantilevers within integrated mechanical platforms.

At the same time, excellent sensitivity was reported in the proteomic field for the specific detection of PSA from a mixture of blood proteins, by using cantilevers functionalized with anti-PSA antibody covalently linked to the cantilever surface.[56] The range of applications grew, including the detection of small chemical molecules such as herbicides and pesticides.[57,58]

Nowadays, the fields and the applications tackled are very extensive, looking for higher sensitivities, integration and packaging, or new information. As an example of the high sensitivities recently achieved, the atrazine pesticide has been detected in concentrations at the picomolar level (concentrations ranging from 4.65 pM to 46.5 μM) within minutes, with high target specificity.[59] In this study, however, no referenced cantilever is used; instead, control experiments are performed to support their results. The aggregation of proteins and the resulting formation of insoluble fibrous protein aggregates have been as well studied through surface stress measurements by using microcantilever sensors.[60] Both, single cantilever and multiple cantilever arrays are used to detect the protein aggregation. The combination of microcantilever sensor with the peptide aptamer technology allows the detection of a specific protein target (CDK2, at 0.08 mg/mL) contained in a complex biological specimen, such as lysate cells.[36]

A novel approach for investigating the mechanisms of antibiotic interactions with mucopeptides was presented by Ndieyira et *al.*[61] The authors have quantified the binding constants for the vamcomycin antibiotic by using differential measurements and reported detection with a sensitivity of 10 nM and at clinically relevant concentrations in blood serum (7 μM of vancomycin in 90% fetal calf serum and 10% sodium phosphate buffer). This work shows the mechanical biosensors as a new technology capable of investigating the antibiotics mechanisms and its modes of action, which could speed up the development of new antibiotics in the battle against drug-resistant bacteria. Enzyme proteins have also been an objective of surface stress studies, because they are a good indicator of disease states and serve as therapeutic targets. As an example, the activity and inhibition of the model proteasa, trypsin, was quantitatively measured with a two-dimensional cantilever array, by injecting various concentrations (1.3×10^{-5} M to 6.5×10^{-7} M) of fibronectin fragment substrate.[35] Fig. 5 shows the cantilever functionalization and blocking of the opposite side, and the surface stress curve as a function of the fibronectin concentration, showing a typical Langmuir isotherm behavior. Another enzyme, the organophosphorus hydrolase (OPH) immobilized over the microcantilever was used for the detection of nerve agents that belong to the organophosphorus compound family (OPs), such as paraoxon, with an approximate detection limit of 10^{-7} M.

Other studies, such as the surface stress associated with conformational changes of proteins, triggered by external signals, have received special attention during the last 4–5 years.[62–65] Bacteriorhodopsin protein is a model system

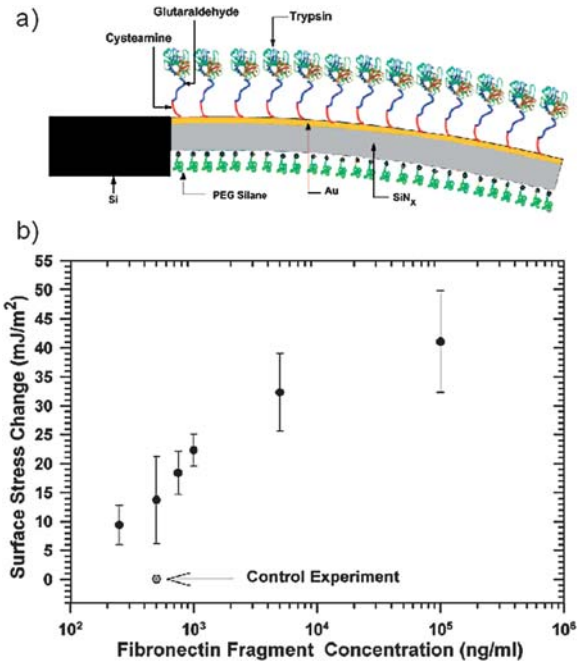

Fig. 5 a) Diagram of the cantilever with the functionalized receptors, using the self-assembled monolayer technique, b) surface stress change *vs.* fibronectin fragment concentration. (Reprinted by permission from Macmillan Publishers Ltd: NanoLetters (ref. 35), copyright 2008).

extensively used for protein conformational changes studies.[66] Changes in the protein size and shape can be photochemically induced, and detected by the surface stress change produced over the cantilever sensor.[63,67] Bálint *et al.* detected the orientation of the protein motion by using polarized light, and estimated the average energy per molecule contributing to the bending to be 195 kT.[67]

Piezoresistive microcantilevers have also demonstrated a very good sensitivity for surface stress detection. Integrated piezoresistive microcantilevers in a CMOS biosensor was proposed to study the DNA hybridization with a sensitivity of 3.5×10^{-5} m/N.[68] In the proteomic field, a concentration of 40 nM of glutathione-*S*-transferase (GST) protein has been detected by using a piezoresistive cantilever array platform with electrical readout.[69]

Dynamic detection

Although most of the biological applications carried out with nanomechanical sensors were originally performed by detecting the cantilever bending, the measurement of the change in the cantilever frequency has currently become a promising method due to its high sensitivity. The delay in achieving a good performance for biological studies under dynamic detection mode is mainly due to the cantilever damping when working in a liquid environment, reducing the quality factor and the system sensitivity, which forced investigations into different strategies to improve the mass-sensing resolution of the sensor. One of the simplest and first approaches was the measurement of the cantilever resonance frequency in air before and after the mass deposition. With this procedure, Ilic *et al.* reported for the first

time the detection of 16 specifically bound *E. coli* cells, which corresponds to a mass of $\sim 6 \times 10^{-12}$ g, by using arrays of microcantilevers covered with specific antibodies.[70] However, this method has the serious inconvenience of drying the biological samples which could lose their natural configuration and properties, having no interest for real sample evaluation. Even with this limitation, the method has been applied during the last years, with high sensitivity, for the detection of single cell,[71] virus,[72] prion proteins,[73] HCV helicase protein[74] and alpha-fetoprotein.[75] Working at high relative humidity, both Gfeller *et al.* and Nugaeva *et al.*, showed the *E. coli* activity and the fungal growth by measuring shifts in the resonance frequency.[76,77]

More recently, working with a silicon microcantilever under a liquid environment, a linear increase in mass sensitivity with the square of the vibration mode number was reported,[78] with a mass resolution of 0.43 pg at mode 7. This method has been recently applied by Braun *et al.* for the evaluation of membrane protein–ligand interactions under physiological conditions.[79] In this work, bacterial virus particles (T5) interacting with their transmembrane receptors (FhuA) were quantitatively detected working at high microcantilever vibration modes (modes 10–15), with an instrument sensitivity of few hundreds of fM, as shown in Fig. 6. The bound mass measured for 3 pM solution was of 8 ng. Working under physiological conditions too, Park *et al.* use "living cantilever arrays" for the mass characterization of single adherent cells, by dividing the vibration spectrum from the cantilever by that of the non-moving substrate,[80] see Fig. 7. The adherent cell was captured and cultured directly on the silicon cantilever. Applications such as mass spectrometry and single-molecule analysis have been already reported due to the high sensitivity reached by using a self-sustained ultrahigh-frequency NEM oscillator (nanocantilevers fixed at both ends).[3,81]

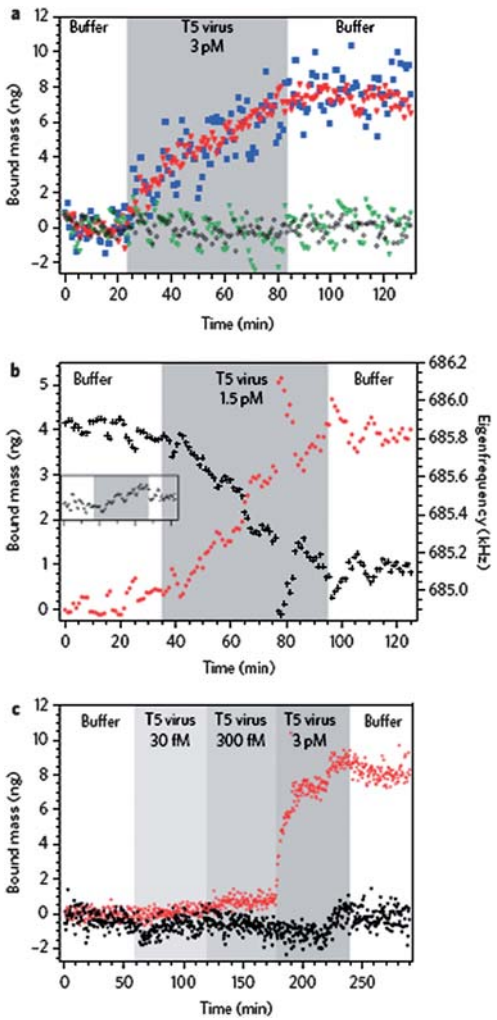

Fig. 6 Docking of T5 phages to FhuA-functionalized cantilevers. a) Mass detection of a 3pM solution of T5 phage. b) Time evolution of the 14th eigenmode of the cantilever for a T5 phage concentration of 1.5 pM. c) Positive and negative control experiments at various concentrations (Reprinted by permission from Macmillan Publishers Ltd: Nature Nanotechnology (ref. 79), copyright 2009).

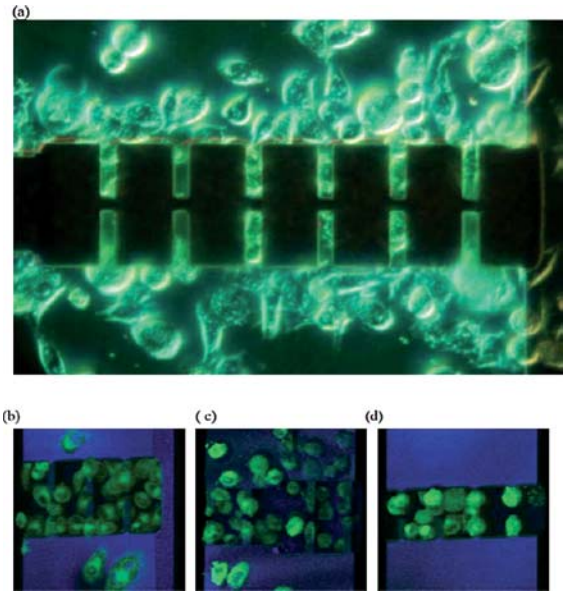

Fig. 7 Cultured cells on the cantilever. Grown HeLa cells in the microfluidic device after 3 days (a), and confocal microscopy images of HeLa cells on (b) 40 μm, (c) 40 and 30 μm and (d) 25 μm long cantilevers. (Reproduced by permission of the Royal Society of Chemistry (ref. 80)).

A completely different approach based on microcantilevers with embedded microchannels was presented by Burg *et al.*, see Fig. 3b.[44] This novel approach eliminates viscous damping by injecting the analyte solution through the cantilever channels, maintaining the cantilever in an air environment, and reporting excellent quality factors of 15000. Binding of goat anti-mouse immunoglobulin-γ, IgG, (0.7 nM), and weighing of individual live bacteria (110 fg for *Escherichia coli* and 150 fg for *Bacillus subtilis*), polystyrene microspheres (91 fg) and gold nanoparticles (10 fg) were performed with these suspended microchannels.

Piezoelectric microcantilevers or piezoelectrically driven cantilevers have been repeatedly used for dynamic applications, for their high amplitude vibration and higher quality factors.[82,83] Self-actuating/sensing piezoelectric microcantilevers were used for real-time monitoring of C reactive protein antigen–antibody interactions in a viscous fluid, with ng resolution. The microcantilevers showed a high quality factor even in viscous liquids with a viscosity comparable to that of blood serum ($Q = 15$ at viscosity of 4.7 cP).[84] A piezoelectric cantilever with dimensions

of 1 × 3 mm has been used for detecting proteins, cells and spores with high sensitivity.[85,86] In spite of the good sensitivity achieved with these macrocantilevers, the size of the sensors could be a limitation for the integration and packaging of a final multi-sensor platform.

Conclusions and outlook

In this review, we have presented the main developments achieved during the last few years in the microcantilever-based biosensing field. It is definitively a fast emerging technology, which has already demonstrated its sensitivity for advanced and complex biological problems. The application range is huge due to the high variety of active layers in contact with the microcantilever that can be used, with very different responses under external stimuli, such as conformational changes, selective swelling, thermal expansion, or changes in the intermolecular forces. The active layer response produces either a change in the cantilever bending or/and in its resonance frequency. The detection of different parameters, such as forces, mass, or stiffness, provides different and complementary information that cannot be obtained with other established label-free biosensors.

The cantilever-based biosensor is still a young technique in constant development. New devices and detection strategies are continuously emerging looking for better understandings, higher sensitivities and simpler system operation. This technique has already demonstrated very extreme limits of detection. A comparative with other established non-labeled biosensors, showing the limit of detection (LOD) for different compounds models is summarized in Table 1. In most of the cases the lower LOD reported is with a cantilever-based biosensor, especially when high mass biological agents are used and the dynamic mode is applied. This table reflects quite well the potential of the mechanical biosensors in the pharmaceutical and medical diagnosis fields.

But there are still some questions that must be addressed to finally develop a highly sensitive and reliable integrated platform able to work with real clinical samples. As in any biosensor, the receptor layer must be specifically assembled for each compound to be detected, and the optimal packaging or optimal pH and ionic strength is different for each case. Covalent immobilization protocols together with blocking agents are usually chosen looking for the final biorecetor layer stability, surface regeneration and avoiding non-specific bindings. Working with cantilevers, the optimization of the receptor layer can be a hard task due to the complex relation between the cantilever response (bending or frequency change) and the forces/mass density acting during the recognition process. Functionalization protocols that had been completely established along the years may need to be modified when working with microcantilevers in terms to enhance the cantilever signal. The surface cleanness and morphology play a very important role in the final detected

Table 1 Comparition of the limits of detection of different biosensors for different biological models

Biosensor principle	Assay principle	Limit of detection	Reference
Pollutant: Atrazine			
SPR	Direct assay (specifically expressed mRNA)	1 ng/L	Lim *et al.*[87]
QCM	Competitive immunoassay	0.025 ng/ml	Prybil *et al.*[88]
	Direct immunoassay	1.5 ng/ml	
Electrochemical	Competitive immunoassay using atrazine-HRP conjugate	6×10^{-3} μg/L	Zacco *et al.*[89]
Nanomechanical	Direct immunoassay (bending)	4.65 pM (1 ng/L)	Suri *et al.*[59]
Cancer marker: PSA			
SPR	Direct immunoassay	300 ng/ml	Besselink *et al.*[90]
	Sandwich immunoassay with colloidal gold nanoparticles	0.15 ng/ml	
QCM	Direct assay based on yeast cells strategy	5 ng/ml	Ding *et al.*[91]
Electrochemical	Sandwich immunoassay	0.25 ng/ml	Sarkar *et al.*[92]
Nanomechanical	Direct immunoassay (static)	0.2 ng/ml	Wu *et al.*[93]
	Direct immunoassay (dynamic)	1 ng/ml	Hwang *et al.*[94]
Bacteria: *Escherichia Coli*			
SPR	Direct immunoassay	10^6 cfu/ml	Subramanian *et al.*[95]
	Sandwich immunoassay	10^3 cfu/ml	
QCM	Direct Lectin mediated detection	7.5×10^2 cfu/ml	Shen *et al.*[96]
Electrochemical	Amperometric based on a double layered configuration	10 cfu/ml	Abu-Rabeah *et al.*[97]
Nanomechanical	In transit (dynamic)	Single cell, 110 fg	Burg *et al.*[44]
Antibiotic: Vancomycin			
SPR	Direct binding	0.3 μM	Tseng *et al.*[98]
	Direct binding	0.31 μM	Cooper *et al.*[99]
QCM	Direct binding	0.6 μM	Tseng *et al.*[98]
Electrochemical	Amperometric detection under HPL conditions	0.5 μg/ml (0.33 μM)	Favetta *et al.*[100]
Nanomechanical	Direct binding (in serum 90%; bending)	10 nM	Ndieyira *et al.*[61]

signal and reproducibility, and must be strictly controlled. Moreover, the use of array of microcantilevers is essential, not only for having a reference cantilever to subtract the effect of non-specific bindings or external effects, but to perform the detection of multianalytes in a single sample. For that reason, further research in the functionalization of arrays of microcantilevers is needed, simplifying the described applied techniques, reducing the time consumption, increasing the reproducibility and providing new routes for multi-analyte detection.

As well, the limitations of the detection systems must be minimized, reducing the noise, thermal fluctuations of the base signal, simplifying the optical alignment and increasing the integration and packaging of the final system. This includes the miniaturization and integration of microfluidics and the detection readout subsystem. For that purpose, the optical waveguide microcantilevers, or the piezoelectric and piezoresistive ones are promising candidates, because of its high integration and not required alignment.

The progress observed during these last few years, when using the static mode and especially with the dynamic mode, must continue. A complete understanding of the surface stress origin is essential to optimize the bending method, reduce the limit of detection and obtain more information about the biological agent under study. In dynamic applications, where the trend is to reduce the cantilever size to increase its sensitivity, the efforts must be addressed towards the noise reduction during the analyte detection, the improvement of the actuation methods and the system resolution for working with viscous samples and the identification of frequency shifts due to mass or stiffness changes.

It is clear that microcantilever-based biosensors have become a competitive technology in the biosensors field, but is still far away from other analytical techniques routinely used in clinical diagnosis laboratories. To that end, the future work should be addressed towards the achievement of a reliable integrated system, able to work with real clinical samples and easy to use for not specialized personnel.

Acknowledgements

Authors acknowledge the financial support from Fundación M. Botín.

References

1 M. Zougagh and A. Rios, *Analyst*, 2009, **134**, 1274–1290.
2 M. Li, W. H. P. Pernice and X. TangH., *Nat. Nanotechnol.*, 2009, **4**, 377–382.
3 X. L. Feng, C. J. White, A. Hajimiri and M. L. Roukes, *Nat. Nanotechnol.*, 2008, **3**, 342–346.
4 B. Ilic, Y. Yang, K. Aubin, R. Reichenbach, S. Krylov and H. G. Craighead, *Nano Lett.*, 2005, **5**, 925–929.
5 J. H. Bak, Y. D. Kim, S. S. Hong, B. Y. Lee, S. R. Lee, J. H. Jang, M. Kim, K. Char, S. Hong and Y. D. Park, *Nat. Mater.*, 2008, **7**, 459–463.
6 M. Watari, J. Galbraith, H. Lang, M. Sousa, M. Hegner, C. Gerber, M. A. Horton and R. A. McKendry, *J. Am. Chem. Soc.*, 2007, **129**, 601–609.
7 J. C. Stachowiak, M. Yue, K. Castelino, A. Chakraborty and A. Majumdar, *Langmuir*, 2006, **22**, 263–268.
8 J. Weissmuller and H. Duan, *Phys. Rev. Lett.*, 2008, **101**, 146102–4.
9 J. Zhang, S. Yu, X. Feng and G. Wang, *J. Appl. Phys.*, 2008, **103**, 093506–6.
10 B. Ilic, H. G. Craighead, S. Krylov, W. Senaratne, C. Ober and P. Neuzil, *J. Appl. Phys.*, 2004, **95**, 3694–3703.
11 S. Kim and K. D. Kihm, *Appl. Phys. Lett.*, 2008, **93**, 081911–3.
12 M. J. Lachut and J. E. Sader, *Phys. Rev. Lett.*, 2007, **99**, 206102–4.
13 P. Lu, H. P. Lee, C. Lu and S. J. O'Shea, *Phys. Rev. B*, 2005, **72**, 085405.
14 A. K. Gupta, P. R. Nair, D. Akin, M. R. Ladisch, S. Broyles, M. A. Alam and R. Bashir, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 13362–13367.
15 S. Bashash, A. Salehi-Khojin, N. Jalili, G. L. Thompson, A. Vertegel, M. Muller and R. Berger, *J. Micromech. Microeng.*, 2009, **19**, 025016.
16 L. Lechuga, J. Tamayo, M. Álvarez, L. Carrascosa, A. Yufera, R. Doldán, E. Peralías, A. Rueda, J. Plaza, K. Zinoviev, C. Domínguez, A. Zaballos, M. Moreno, C. Martínez-A, D. Wenn, N. Harris, C. Bringer, V. Bardinal, T. Camps, C. Vergnenègre, C. Fontaine, V. Díaz and A. Bernad, *Sens. Actuators, B*, 2006, **118**, 2–10.
17 H. P. Lang, R. Berger, C. Andreoli, J. Brugger, M. Despont, P. Vettiger, C. Gerber, J. K. Gimzewski, J. P. Ramseyer, E. Meyer and H. Guntherodt, *Appl. Phys. Lett.*, 1998, **72**, 383–385.
18 Min Yue, H. Lin, D. Dedrick, S. Satyanarayana, A. Majumdar, A. Bedekar, J. Jenkins and S. Sundaram, *J. Microelectromech. Syst.*, 2004, **13**, 290–299.
19 G. Shekhawat, S. Tark and V. P. Dravid, *Science*, 2006, **311**, 1592–1595.
20 K. Zinoviev, C. Dominguez, J. Plaza, V. Busto and L. Lechuga, *J. Lightwave Technol.*, 2006, **24**, 2132–2138.
21 K. Zinoviev, C. Dominguez, J. A. Plaza and L. M. Lechuga, *Appl. Phys. Lett.*, 2008, **92**, 011908–3.
22 K. Zinoviev, C. Dominguez, J. A. Plaza, V. Cadarso and L. M. Lechuga, *Appl. Opt.*, 2006, **45**, 229–234.
23 J. A. Plaza, K. Zinoviev, G. Villanueva, M. Alvarez, J. Tamayo, C. Dominguez and L. M. Lechuga, *Appl. Phys. Lett.*, 2006, **89**, 094109–3.
24 C. A. Savran, S. M. Knudsen, A. D. Ellington and S. R. Manalis, *Anal. Chem.*, 2004, **76**, 3194–3198.
25 A. Loui, F. Goericke, T. Ratto, J. Lee, B. Hart and W. King, *Sens. Actuators, A*, 2008, **147**, 516–521.
26 Tao Xu, Ruisheng Chang, M. Bachman, and Guann-Pyng Li, in *Sensors, 2005 IEEE*, 2005, p. 4 pp.
27 M. Nordstrom, D. Zauner, A. Boisen and J. Hubner, *J. Lightwave Technol.*, 2007, **25**, 1284–1289.
28 N. Siwak, Xiao Zhu Fan, D. Hines, S. Kanakaraju, N. Goldsman and R. Ghodssi, *J. Microelectromech. Syst.*, 2009, **18**, 103–110.
29 C. Lee, J. Thillaigovindan, C. Chen, X. T. Chen, Y. Chao, S. Tao, W. Xiang, A. Yu, H. Feng and G. Q. Lo, *Appl. Phys. Lett.*, 2008, **93**, 113113–3.
30 R. Desikan, I. Lee and T. Thundat, *Ultramicroscopy*, 2006, **106**, 795–799.
31 V. Tabard-Cossa, M. Godin, I. J. Burgess, T. Monga, R. B. Lennox and P. Grutter, *Anal. Chem.*, 2007, **79**, 8136–8143.
32 S. Singamaneni, M. LeMieux, H. Lang, C. Gerber, Y. Lam, S. Zauscher, P. Datskos, N. Lavrik, H. Jiang, R. Naik, T. Bunning and V. Tsukruk, *Adv. Mater.*, 2008, **20**, 653–680.
33 L. L. Norman and A. Badia, *J. Am. Chem. Soc.*, 2009, **131**, 2328–2337.
34 E. Mauriz, A. Calle, J. Manclús, A. Montoya, A. Hildebrandt, D. Barceló and L. Lechuga, *Biosens. Bioelectron.*, 2007, **22**, 1410–1418.
35 D. A. Raorane, M. D. Lim, F. F. Chen, C. S. Craik and A. Majumdar, *Nano Lett.*, 2008, **8**, 2968–2974.
36 W. Shu, S. Laurenson, T. P. Knowles, P. Ko Ferrigno and A. A. Seshia, *Biosens. Bioelectron.*, 2008, **24**, 233–237.
37 F. Zhou, W. Shu, M. E. Welland and W. T. S. Huck, *J. Am. Chem. Soc.*, 2006, **128**, 5326–5327.
38 F. Zhou, P. M. Biesheuvel, E. Choi, W. Shu, R. Poetes, U. Steiner and W. T. S. Huck, *Nano Lett.*, 2008, **8**, 725–30.
39 H. P. Lang, J. Ramseyer, W. Grange, T. Braun, D. Schmid, P. Hunziker, C. Jung, M. Hegner and C. Gerber, *J. Phys. Conf. Ser.*, 2007, **61**, 663.
40 Y. Lin, M. McConney, M. LeMieux, S. Peleshanko, C. Jiang, S. Singamaneni and V. Tsukruk, *Adv. Mater.*, 2006, **18**, 1157–1161.
41 G. Oliviero, P. Bergese, G. Canavese, M. Chiari, P. Colombi, M. Cretich, F. Damin, S. Fiorilli, S. L. Marasso, C. Ricciardi, P. Rivolo and L. E. Depero, *Anal. Chim. Acta*, 2008, **630**, 161–167.

42 P. Belaubre, M. Guirardel, G. Garcia, J. B. Pourciel, V. Leberre, A. Dagkessamanskaia, E. Trevisiol, J. M. Francois and C. Bergaud, *Appl. Phys. Lett.*, 2003, **82**, 3122–3124.
43 A. Bietsch, J. Zhang, M. Hegner, H. P. Lang and C. Gerber, *Nanotechnology*, 2004, **15**, 873.
44 T. P. Burg, M. Godin, S. M. Knudsen, W. Shen, G. Carlson, J. S. Foster, K. Babcock and S. R. Manalis, *Nature*, 2007, **446**, 1066–1069.
45 Y. Huang, H. Liu, K. Li, Y. Chen, Q. Zhang and X. Wu, *Sens. Actuators, A*, 2008, **148**, 329–334.
46 F. Huber, H. P. Lang, M. Hegner, M. Despont, U. Drechsler and C. Gerber, *Rev. Sci. Instrum.*, 2008, **79**, 086110–3.
47 M. L. Sushko, J. H. Harding, A. L. Shluger, R. A. McKendry and M. Watari, *Adv. Mater.*, 2008, **20**, 3848–3853.
48 H. Butt, *J. Colloid Interface Sci.*, 1996, **180**, 251–260.
49 A. M. Moulin, S. J. O'Shea, R. A. Badley, P. Doyle and M. E. Welland, *Langmuir*, 1999, **15**, 8776–8779.
50 J. Fritz, M. K. Baller, H. P. Lang, H. Rothuizen, P. Vettiger, E. Meyer, H. -J. Güntherodt, C. Gerber and J. K. Gimzewski, *Science*, 2000, **288**, 316–318.
51 K. M. Hansen, H. F. Ji, G. Wu, R. Datar, R. Cote, A. Majumdar and T. Thundat, *Anal. Chem.*, 2001, **73**, 1567–1571.
52 R. McKendry, J. Zhang, Y. Arntz, T. Strunz, M. Hegner, H. P. Lang, M. K. Baller, U. Certa, E. Meyer, H. Güntherodt and C. Gerber, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 9783–9788.
53 G. Wu, H. Ji, K. Hansen, T. Thundat, R. Datar, R. Cote, M. F. Hagan, A. K. Chakraborty and A. Majumdar, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 1560–1564.
54 M. Alvarez, L. G. Carrascosa, M. Moreno, A. Calle, A. Zaballos, L. M. Lechuga, C. Martinez-A and J. Tamayo, *Langmuir*, 2004, **20**, 9663–9668.
55 H. P. Lang, M. Hegner, E. Meyer, and C. Gerber, 2002, 13, p. R29.
56 G. Wu, R. H. Datar, K. M. Hansen, T. Thundat, R. J. Cote and A. Majumdar, *Nat. Biotechnol.*, 2001, **19**, 856–860.
57 R. Raiteri, M. Grattarola, H. Butt and P. Skládal, *Sens. Actuators, B*, 2001, **79**, 115–126.
58 M. Alvarez, A. Calle, J. Tamayo, L. M. Lechuga, A. Abad and A. Montoya, *Biosens. Bioelectron.*, 2003, **18**, 649–653.
59 C. R. Suri, J. Kaur, S. Gandhi and G. S. Shekhawat, *Nanotechnology*, 2008, **19**, 235502.
60 T. P. J. knowles, W. Shu, F. Huber, H. P. Lang, C. Gerber, C. M. Dobson and M. E. Welland, *Nanotechnology*, 2008, **19**, 384007.
61 J. W. Ndieyira, M. Watari, A. D. Barrera, D. Zhou, M. Vogtli, M. Batchelor, M. A. Cooper, T. Strunz, M. A. Horton, C. Abell, T. Rayment, G. Aeppli and R. A. McKendry, *Nat. Nanotechnol.*, 2008, **3**, 691–696.
62 R. Mukhopadhyay, V. V. Sumbayev, M. Lorentzen, J. Kjems, P. A. Andreasen and F. Besenbacher, *Nano Lett.*, 2005, **5**, 2385–2388.
63 T. Braun, N. Backmann, M. Vögtli, Alex, E. Bietsch, A. Engel, H. Lang, C. Gerber and M. Hegner, *Biophys. J.*, 2006, **90**, 2970–2977.
64 W. Shu, D. Liu, M. Watari, C. K. Riener, T. Strunz, M. E. Welland, S. Balasubramanian and R. A. McKendry, *J. Am. Chem. Soc.*, 2005, **127**, 17054–17060.
65 H. Ji, H. Gao, K. R. Buchapudi, X. Yang, X. Xu and M. K. Schulte, *Analyst*, 2008, **133**, 434–443.
66 K. Voïtchovsky, S. A. Contera and J. Ryan, *Biophys. J.*, 2007, **93**, 2024–2037.
67 Z. Balint, G. A. Vegh, A. Popescu, M. Dima, C. Ganea and G. Varo, *Langmuir*, 2007, **23**, 7225–7228.
68 S. Yang, C. Chang, T. Yin and P. Kuo, *Sens. Actuators, B*, 2008, **130**, 674–681.
69 V. Dauksaite, M. Lorentzen, F. Besenbacher and J. Kjems, *Nanotechnology*, 2007, **18**, 125503.
70 B. Ilic, D. Czaplewski, H. G. Craighead, P. Neuzil, C. Campagnolo and C. Batt, *Appl. Phys. Lett.*, 2000, **77**, 450–452.
71 B. Ilic, D. Czaplewski, M. Zalalutdinov, H. G. Craighead, P. Neuzil, C. Campagnolo and C. Batt, in *The 45th international conference on electron, ion, and photon beam technology and nanofabrication*, AVS, Washington, DC (USA), 2001, vol. 19, pp. 2825–2828.
72 B. Ilic, Y. Yang and H. G. Craighead, *Appl. Phys. Lett.*, 2004, **85**, 2604–2606.
73 M. Varshney, P. S. Waggoner, C. P. Tan, K. Aubin, R. A. Montagna and H. G. Craighead, *Anal. Chem.*, 2008, **80**, 2141–2148.
74 K. S. Hwang, S. Lee, K. Eom, J. H. Lee, Y. Lee, J. H. Park, D. S. Yoon and T. S. Kim, *Biosens. Bioelectron.*, 2007, **23**, 459–465.
75 Y. Liu, X. Li, Z. Zhang, G. Zuo, Z. Cheng and H. Yu, *Biomed. Microdevices*, 2009, **11**, 183–191.
76 K. Y. Gfeller, N. Nugaeva and M. Hegner, *Appl. Environ. Microbiol.*, 2005, **71**, 2626–2631.
77 N. Nugaeva, K. Y. Gfeller, N. Backmann, H. P. Lang, M. Düggelin and M. Hegner, *Biosens. Bioelectron.*, 2005, **21**, 849–856.
78 M. K. Ghatkesar, V. Barwich, T. Braun, J. Ramseyer, C. Gerber, H. P. Lang, U. Drechsler and M. Despont, *Nanotechnology*, 2007, **18**, 445502.
79 T. Braun, M. K. Ghatkesar, N. Backmann, W. Grange, P. Boulanger, L. Letellier, H. Lang, A. Bietsch, C. Gerber and M. Hegner, *Nat. Nanotechnol.*, 2009, **4**, 179–185.
80 K. Park, J. Jang, D. Irimia, J. Sturgis, J. Lee, J. P. Robinson, M. Toner and R. Bashir, *Lab Chip*, 2008, **8**, 1034–1041.
81 A. K. Naik, M. S. Hanay, W. K. Hiebert, X. L. Feng and M. L. Roukes, *Nat. Nanotechnol.*, 2009, **4**, 445–450.
82 A. Salehi-Khojin, S. Bashash, N. Jalili, M. Muller and R. Berger, *J. Appl. Phys.*, 2009, **105**, 013506–8.
83 S. Shin, J. P. Kim, S. J. Sim and J. Lee, *Appl. Phys. Lett.*, 2008, **93**, 102902–3.
84 T. Y. Kwon, K. Eom, J. H. Park, D. S. Yoon, T. S. Kim and H. L. Lee, *Appl. Phys. Lett.*, 2007, **90**, 223903–3.
85 D. Maraldo, K. Rijal, G. Campbell and R. Mutharasan, *Anal. Chem.*, 2007, **79**, 2762–2770.
86 J. McGovern, W. Y. Shih, R. Rest, M. Purohit, Y. Pandya and W. Shih, *Analyst*, 2008, **133**, 649–654.
87 T. Lim, M. Oyama, K. Ikebukuro and I. Karube, *Anal. Chem.*, 2000, **72**, 2856–2860.
88 J. Pribyl, M. Hepel, J. Halámek and P. Skládal, *Sens. Actuators, B*, 2003, **91**, 333–341.
89 E. Zacco, R. Galve, M. P. Marco, S. Alegret and M. I. Pividori, *Biosens. Bioelectron.*, 2007, **22**, 1707–1715.
90 G. A. J. Besselink, R. P. H. Kooyman, P. J. H. J. van Os, G. H. M. Engbers and R. B. M. Schasfoort, *Anal. Biochem.*, 2004, **333**, 165–173.
91 Y. Ding, H. Lu, G. Shi, J. Liu, G. Shen and R. Yu, *Biosens. Bioelectron.*, 2008, **24**, 228–232.
92 P. Sarkar, P. S. Pal, D. Ghosh, S. J. Setford and I. E. Tothill, *Int. J. Pharm.*, 2002, **238**, 1–9.
93 G. Wu, R. H. Datar, K. M. Hansen, T. Thundat, R. J. Cote and A. Majumdar, *Nat. Biotechnol.*, 2001, **19**, 856–860.
94 K. S. Hwang, J. H. Lee, J. Park, D. S. Yoon, J. H. Park and T. S. Kim, *Lab Chip*, 2004, **4**, 547–552.
95 A. Subramanian, J. Irudayaraj and T. Ryan, *Biosens. Bioelectron.*, 2006, **21**, 998–1006.
96 Z. Shen, M. Huang, C. Xiao, Y. Zhang, X. Zeng and P. G. Wang, *Anal. Chem.*, 2007, **79**, 2312–2319.
97 K. Abu-Rabeah, A. Ashkenazi, D. Atias, L. Amir and R. S. Marks, *Biosens. Bioelectron.*, 2009, **24**, 3461–3466.
98 M. Tseng, Y. Chang and Y. Chu, *Anal. Biochem.*, 2007, **371**, 1–9.
99 M. A. Cooper, M. T. Fiorini, C. Abell and D. H. Williams, *Bioorg. Med. Chem.*, 2000, **8**, 2609–2616.
100 P. Favetta, J. Guitton, C. Dufresne and J. Bureau, *J. Chromatogr. B*, 2001, **751**, 377–382.

