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Surface stress-based biosensors

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ABSTRACT

Surface stress-based biosensors, as one kind of label-free biosensors, have attracted lots of attention in the process of information gathering and measurement for the biological, chemical and medical application with the development of technology and society. This kind of biosensors offers many advantages such as short response time (less than milliseconds) and a typical sensitivity at nanogram, picoliter, femtojoule and attomolar level. Furthermore, it simplifies sample preparation and testing procedures. In this work, progress made towards the use of surface stress-based biosensors for achieving better performance is critically reviewed, including our recent achievement, the optimally circular membrane-based biosensors and biosensor array. The further scientific and technological challenges in this field are also summarized. Critical remark and future steps towards the ultimate surface stress-based biosensors are addressed.

Contents

1. Introduction

"Biological micro-electromechanical systems" (BioMEMS) is a special class of MEMS where biological matter is manipulated for analysis and measurement of its activity, characterizations under any class of scientific study (Wang and Soper, 2007). The BioMEMS-based technology has achieved exciting development in recent decades. Biosensors, especially as portable Point of Care (PoC) systems, attract considerable attention with the development of the BioMEMS technology and miniaturized application. As the increasing demand in the areas—genetics, diagnostics, drug discovery, environment, industrial monitoring and quality control (Satyanarayana, 2005), the need for high throughput label-free

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multiplexed sensors for biological sensing has increased in last decade.

Surface stress-based biosensors, as one new technology of micro-scale and label-free system, have immense potential to satisfy the demand for better sensor quality and have been investigated extensively in recent years. This kind of sensors use a balance of free energy change and offer a universal platform for chemical and biological sensing (Satyanarayana, 2005). They have many advantages over other sensors, such as short response time (less than milliseconds) and a typical sensitivity at nanogram, picoliter, femtojoule and attomolar level. Surface stress-based biosensors also could be made into paralleled array, enabling multiple sensing simultaneously. Furthermore, as one kind of label-free sensors, it simplifies sample preparation and testing procedures compared to methods that involve labeling, e.g., fluorescent and others (Sang, 2011).

Microcantilevers (Yin et al., 2013; Johnson and Mutharasan, 2012; Zimmermann et al., 2008; Hanse and Thundat, 2005) and micromembranes (Zhang et al., 2013; Tsouti et al., 2010; Sang, 2010; Wilson et al., 2009; Cha et al., 2008) are two kinds of sensitive element of the surface stress-based biosensors. Lots of researches have been conducted in the field of surface stress-based biosensors. Antibodies (Tseng et al., 2012; Binnig et al., 1986), cell growth (Liu et al., 2013), pH changes (Willemsen et al., 2000), hybridization of DNA (Hansen et al., 2001; Wu et al., 2001b; Florin et al., 1994) can be detected using the surface stress-based microcantilever biosensors. In order to get more reliable interaction measurement, some researchers tried to use polyethylene glycol (PEG) as the inert coatings in the backside of cantilever detection system for backside passivation (Raorane et al., 2008a; Backmann et al., 2005; Yue et al. 2004). This method can reduce the backside physisorption and increase the signal-to-noise ratio. Surface stress-based micromembrane biosensors have been studied and developed by other researchers to further improve the performance of biosensors (Zhang et al., 2013; Tsouti et al., 2011; Sang and Witte, 2010a; Tsouti et al., 2010; Kang et al., 2010; Cha et al., 2008; Satyanarayana et al., 2006). This kind of surface stress-based biosensors can further improve the signal-to-noise ratio because the membrane backside will not contact with the test solution and so there is no backside physisorption essentially. Different membrane materials and structures have also been studied to improve the characteristics of surface stress-based micromembrane biosensors.

Surface stress-based biosensors must also associate with some detection methods (for example, optical detection, piezoresistive detection, capacitive detection) to measure the effective information obtained from the analytes. Therefore, the development of detection technology has a strong impact on the evolution of surface stress-based biosensors.

This review is focusing on recent development in the field of surface stress-based biosensors. Fundamental aspects, such as the mechanism of surface stress production, the functional technology, the transducer technology, the detection technology, and the current situation and development are summarized. This review also summarizes the further scientific and technological challenges in this particular field. We hope this review would be helpful for better understanding the importance of surface stress in developing the next generation of biosensors.

2. Theory of surface stress

Surface stress was first defined by Josiah Willard Gibbs as the amount of reversible work per unit area needed to elastically stretch a pre-existing surface (Gibbs and Green, 1906). Origin of surface stress could be understood from the chemical bonding of atoms at the surface. At the most basic level, surface stress arises when surface atoms or thin films undergo some dynamic microstructural process resulting in a change in density (Monrudee, 2008). If bond strengths between surface atoms are stronger than that between sub-surface atoms (in the bulk), a tensile surface stress generates by the attractive forces between these surface atoms, resulting in a concave surface curvature (Monrudee, 2008). In contrast, if surface atoms tend to repel each other, a compressive surface stress is induced, resulting in a convex surface curvature, as Fig. 1 shows.

In general, the origins of surface stress can be quite different, from atomistic interactions between surface atoms, to inter molecular interactions between species adsorbed on a surface, to the expansion of a deposited thin film arising from temperature changes or phase transitions (Godin, 2004). Electron diffraction (Wasserman and Vermaak, 1972; Mays et al., 1968) of nanoparticles has been studied for the measure of the absolute surface stress. The investigation of surface reconstruction, interfacial mixing, and self-organization at solid surfaces have aroused interest in the study of surface stress (Tartaglino et al., 2002; Pohl et al., 1999; Bach et al., 1997; Berger et al., 1997; Tromp et al., 1992).

Surface stress is formally treated in tensor form via the surface stress tensor σ_{ij}. It is useful to consider the Shuttleworth equation (Shuttleworth, 1950) defining surface stress in term of surface energy, γ

$$\sigma_{ij} = \gamma\delta_{ij} + \partial\gamma/\partial\varepsilon_{ij} \quad (1)$$

Where δ_{ij} is the Kronecker delta and ε_{ij} is the elastic strain tensor. For a liquid surface, there is no resistance to plastic deformation, so the second term of Eq. (1) vanishes, surface stress and surface energy are equal. For solid interfaces, the second term on the right-hand side of Eq. (1) is not equal to zero. Qualitatively, surface energy is related to the changes in energy during plastic formation of a surface area. Therefore surface stress is related to changes in energy during elastic stretching of a pre-existing surface.

The surface stress also exists between biological molecules, cells and some special functional materials, which can be used in the biosensors for biological and medical research. When bio/chemical reactions occur preferentially on one surface of the surface stress-based biosensors, changes in intermolecular forces can create a surface stress that alters the curvature of the mechanical sensing element. The range of surface stress in such reactions was reported to be from 5 mJ/m^2 to 50 mJ/m^2 (Yue et al., 2004; Yue, 2004; Wu et al., 2001a; Wu et al., 2001b) or as high as 200 mJ/m^2 (Berger et al., 1997) or even 900 mJ/m^2 (Marie et al., 2002).

3. Surface stress-based biosensor

A biosensor is a device for the detection of an analyte that combines a biological component with a physicochemical detector system (McNaught and Wilkison, 1997). One concept of surface stress-based biosensors encompasses two main features: the sensitive biological element which is a chemically receptive or selective layer, and the transducer or the detector element (Fig. 2). In addition,

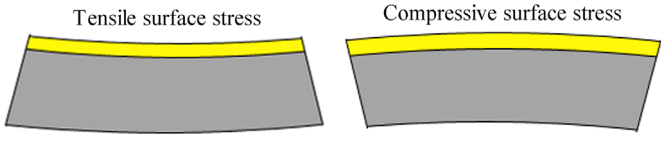

Fig. 1. A tensile surface stress (positive surface stress) contracts the top surface of a thin plate inducing a concave curvature. A compressive surface stress (negative surface stress) expands the top surface of a thin plate inducing a convex curvature. Image adapted from Godin (2004).

a theoretical biosensor always have an associated transporter used for the transport of analytes, and a signal processor used to display the results in a user-friendly way.

The selective layer provides specific binding sites for the target analyte of interest, such as molecules, proteins and cells. The transducer layer transforms the surface stress signal induced by the interaction between analytes and selective layer into another signal which can be measured and quantified more easily through physico-chemical, optical, piezoelectric, electro-chemical ways, etc. (Sang, 2010).

3.1. Transporter of analyters

The analytes in biosensors are always geometrically constrained to a small size, typically sub-millimeter. It has some features, small volume (nl, pl, fl) and characteristic sizes, low energy consumption, and micro-domain effects, for example. Microfluidic platforms can mimic conventional analyte handling techniques which performs to enable both medical research and healthcare advances. The miniaturized microfluidic devices or components have been termed "micro-total analysis systems" (μTAS) or "biochips" (Su et al., 2006). These autonomous platforms consisted of microfluidics and biosensor have attracted considerable research interest, because they provide the opportunity for fabricating a highly integrated system able to perform all necessary processing steps required for the specific application. Fig. 3 presents an application of microfluidic devices in membrane biosensor (Varshney et al., 2007).

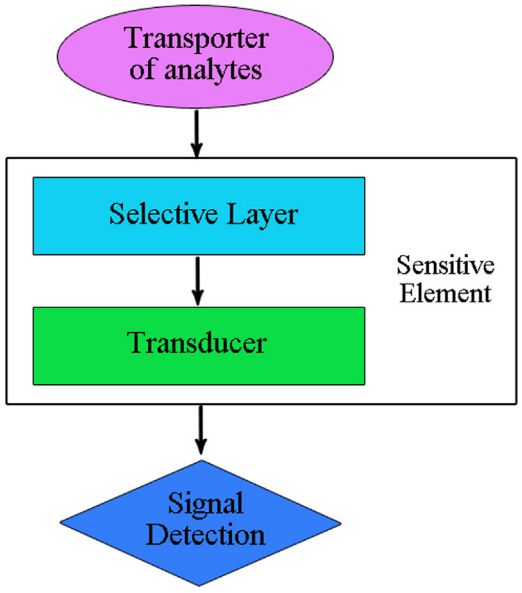

Fig. 2. Generalized schematic representation of a biological sensor. Image adapted from Sang (2010).

Microfluidic device is a multidisciplinary field intersecting engineering, physics, chemistry, microtechnology and biotechnology (Kuznetsov, 2010). These applications are attractive because of the potential that such systems allow faster analysis of biological material. Further they can reduce the requirement of reagent amount and the number of processing steps. In addition, miniaturization of such systems can result in higher repeatability and precision of analysis, lower power consumption, and the potential to create portable diagnostic tools for on-site analysis. These advantages result in not only time and cost savings for diagnostic tests, but can also be life saving in time-critical environments such as critical medical diagnostics or biowarfare pathogen detection. Microfluidics is a relatively mature technology. There are many books and papers about it, so we will not review verbosely here.

3.2. Sensitive element

3.2.1. Selective layer

The sensor's selectivity, i.e. specificity of the sensor to an especial analyte, is determined by the selective layer of the biosensors. The selective layer can be designed employing principles of molecular and biomolecular recognition, for example, antigen-antibody binding (i.e. any chemicals, bacteria, viruses, or pollen binding to a specific protein) (Tabard-Cossa, 2005). Otherwise, very specific surface selectivity can be achieved using molecular self-assembled monolayers (SAMs) as the sensing layers assembled on the surface of biosensor sensitive element. It has been studied by many researchers (Tseng et al., 2012; Horikawa et al., 2011; Ji and Armon, 2010; Sang, 2010; Yang and Chang, 2010; Boisen and Thundat, 2009; Arntz et al., 2003; Subramanian et al., 2002; Schwartz, 2001; Fritz et al., 2000;

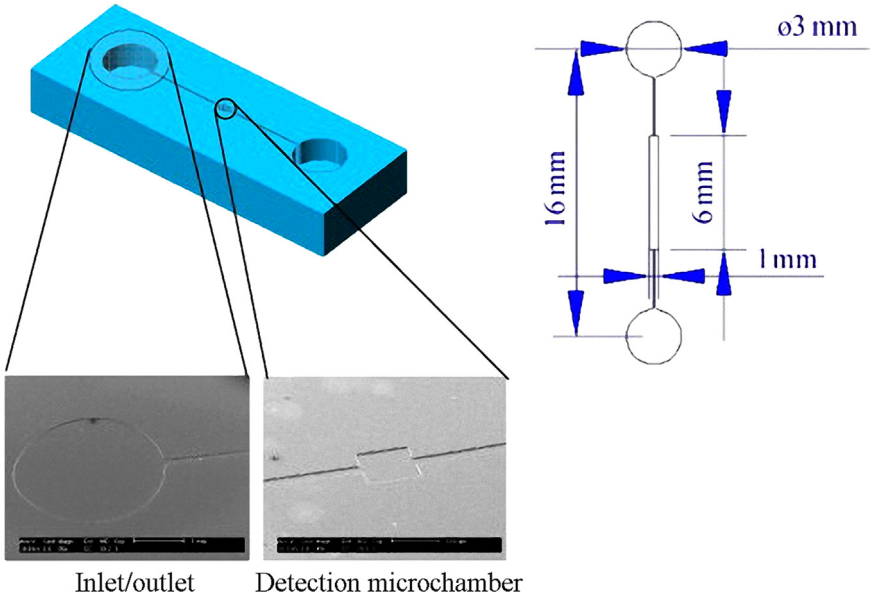

Fig. 3. A microfuildic devices in membrane biosensor. The microfluidic includes inlet, microchannel and outlet. The pressure method is used to load solution and cells in the microfluidics. The cavity is opened to the ambient air by another air outlet to reduce the fluctuation. The microfluidic material wafers are glass. Image from Varshney et al. (2007).

Schreiber, 2000; Kondoh et al., 1999; Poirier, 1997; DiMilla et al., 1994; Prime and Whitesides, 1993). This review does not discuss the technology because surface functionalization, specificity and selectivity of biological receptors are too complex matters. Readers can refer to corresponding references.

3.2.2. Transducer layer

A surface stress-based transducer structure has two basic layers: a submaterial layer (such as polydimethylsiloxane (PDMS), silicon, aluminum nitride, silicon nitride, polymethylmethacrylate (PMMA) etc.) and a contact layer, i.e. gold layer. Fig. 4 illustrates the schematic diagram of surface stress-based transducer. The gold layer on the top of the base layer is essential to form the self-assembled monolayers (Widge et al., 2007), which is necessary to improve biocompatibility and provide sufficient bending stiffness for selective layer (Sang and Witte, 2010a).

The transducer has multifarious structures based on different excogitation and signal detection methods. Cantilever and membrane are two commonly used transducer structures of the surface stress-based biosensors. The physical transducer often determines the limits of detection attainable although the chemical layer (i.e. selective layer) also imposes some limitations on the performance of biosensors (Tabard-Cossa, 2005). So the search for new design scheme of transducer has been constantly stimulating.

3.2.3. Cantilever transducer

The surface stress-based cantilever biosensors have been found a growing interest in the biological detection and quantification in the last decade. Cantilevers are used as the sensitive elements of biosensors due to their high sensitivity and brief structure. In most cantilever-based sensing applications, one surface of the cantilever beam is rendered sensitive to a specific target molecule of interest, while the opposing surface is chemically passivated. When these target molecules interact with the sensitized surface of the cantilever, a surface stress can be induced. The difference of surface stress between the sensitive surface and the passive surface can result in a measurable mechanical deflection. Fig. 5 schematically shows a bending cantilever induced by the molecular surface stress.

The rectangular-shaped cantilever is usually chosen as the sensitive element of the microcantilever biosensors (Tabard-Cossa et al., 2005; Yue et al., 2004; Marie et al., 2002; Wu et al., 2001b).

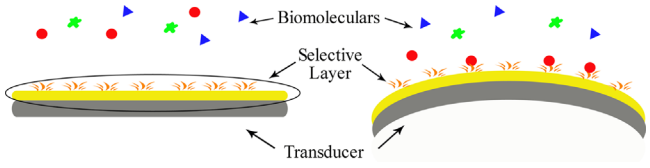

Fig. 4. The surface stress-based sensitive element.

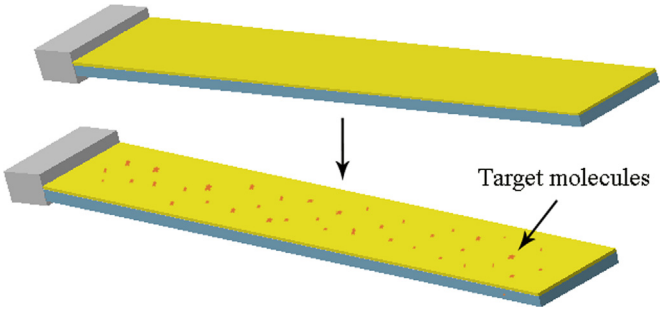

Fig. 5. A cantilever beam acts as the sensitive element of biological sensor by transducing the surface stress induced by the adsorption of target molecules into the measurable deflection.

In general, the lateral size and shape of the cantilever do not directly influence the sensitivity of the sensor because the surface-stress change to be detected is in principle isotropic and uniform over the functionalized surface. Particularly, this is in contrast to similar sensors based on optical read-out (Hanse and Thundat, 2005; Kwak et al., 2003; Wu et al., 2001a; Moulin et al., 2000), which measures the tip deflection of the cantilever. The tip deflection is quadratic to the length of the cantilever for a small and uniform bending. The surface stress change is constant over the active surface area and independent of the width. Therefore, long cantilevers are usually preferred for optical read-out schemes (Zimmermann et al., 2008). In summary, the cantilever length and width should be large, compared to the cantilever thickness for the surface stress to be effectively transferred into the transducer sensitive elements.

Triangular-shaped cantilever is another choice for microcantilever biosensors as the sensitive element. Generally, silicon cantilevers which are more rigid than commonly-used silicon-nitride cantilevers can be micro-fabricated with sufficiently low spring constants for special chemical/biological sensing applications (Drelich et al., 2004). Although the triangular silicon nitride cantilevers are more difficult to calibrate compared to most other commercially available single-crystal silicon, they have a lower spring constant ($k \approx 0.01$ N/m) which makes them more sensitive. Furthermore, the triangular shape makes them easier to reproducibly align the laser used for deflection sensing (Kailas et al., 2009; Alcaraz et al., 2003; Vié et al., 2000). Fig. 6 shows a commercially-available triangular-shaped silicon-nitride cantilever (Raiteri et al., 2002).

However, surface stress-based cantilever biosensors are not free from problems which include limitations imposed by the bulky size of the optical detection system (Seo et al., 2007; Lee et al., 2004), loss of signal due to the severe bending of the cantilever (Grogan et al. (2002); Raiteri et al., 2001), and the limited dynamic range. Moreover, the cantilever geometry is not the best for sensing in liquid media, which is most common in biological applications, because the entire cantilever is immersed in the fluid and non-specific adsorption at the back side of the

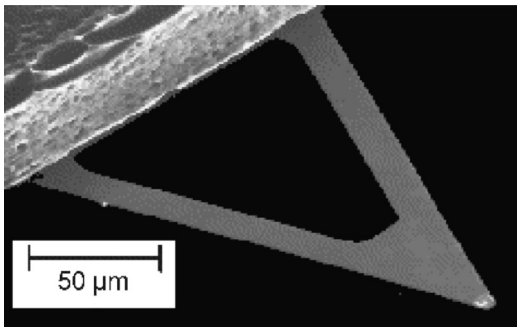

Fig. 6. A picture of commercially-available triangular-shaped silicon-nitride cantilever. Image adapted from Raiteri et al. (2002).

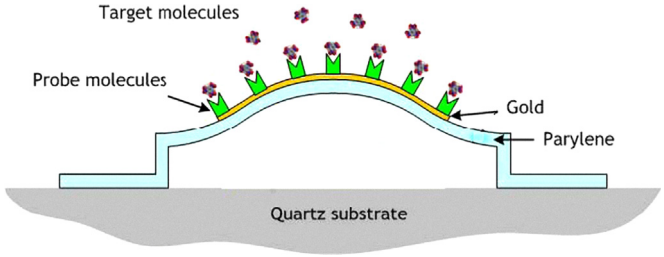

Fig. 7. Structure of surface stress-based membrane biosensor element. Image adapted from Lim et al. (2007).

cantilever could drastically reduce the signal-to-noise ratio (SNR) (Satyanarayana, 2005) and induce the long term drift. In some specific cases, the cantilever-based technique could not reflect the true state of the biologics when taking measurements because biological materials generally conform to their native state in a liquid environment. The risk of breaking the cantilever also exists when transferred it from one place to another (Campbell, 2006). Hence, the cantilever-based surface stress biosensors are not the best choice in most cases. The better transducers have being developed, such as membrance transducer.

3.2.4. Membrane transducer

Membrane is another configuration of a sensitive mechanical element for detecting surface stress changes. One conceptual design of the parylene micromembrane sensor is illustrated in Fig. 7 (Lim et al., 2007). In general, the membrane is fixed on all four sides and is suspended over a substrate. A part of the membrane surface is modified to selectively attach probe molecules to the surface. When selective chemical/biochemical reactions occur between the target and probe molecules on the sensor surface, the changes in the intermolecular forces induce a surface stress change which causes the membrane to curve.

Membranes offer many advantages over cantilevers, for example, sample isolation from the detection system and easy electronic readout, such as capacitive detection (Chatzipetrou et al., 2013; Satyanarayana, 2005; Chatzandroulis et al., 2004). But, for a given sensor dimension, membranes are less compliant than cantilevers which results in lower sensitivity. The sensitive problem can be solved by using low stiffness materials like Parylene (Satyanarayana et al., 2006), SU8, PMMA and PDMS (Zhang et al., 2013; Sang, 2010; Cha et al., 2008) instead of silicon (Tsouti et al., 2010), silicon oxide (Astié et al., 2000) or silicon nitride (Wilson et al., 2009) based materials.

The deflection characteristics of membranes built with various substrates were studied by Sang and Witte (2009). They found that PDMS is a particular material for the surface stress-based membrane, owing to its lower stiffness than silicon, aluminum nitride, silicon nitride and PMMA. Young's modulus of bulk PDMS usually falls within 12 kPa–2.5 Mpa (Sang and Witte, 2010b; Brown et al., 2005; Eddington et al., 2003; Gray et al., 2003;Tan et al., 2003; Armani1 et al., 1999) depending on the processing conditions. Furthermore, PDMS has the characteristic of biocompatibility and ease of processing (Sang and Witte, 2010b; DeSilva et al., 2006; DuRoure et al., 2005; Peterson et al., 2005; Ostuni et al., 2000). So surface stress-based PDMS membrane biosensors have being investigated in recent years. In 2004, Yue proved that overall deflection should be dominated by the thin gold film rather than the thick PDMS membrane because of the larger Young's modulus (Yue, 2004). Many experiences of fabrication show that the thin film of gold would wrinkle in an uncontrollable way when unsupported. Therefore researchers proposed that the PDMS is necessary to form the dome shaped membrane during the fabrication process. Detailed characteristics of PDMS as the substrate membrane have been systematically studied by Sang (2010). These biosensors have also been successfully fabricated through conquering many challenges, e.g., integration of PDMS processing with conventional micro-fabrication processes and the fabrication of perfect PDMS thin film (Sang, 2010). Based on our research, the micro-membrane sensor shows low elasticity, better biological and chemical resistance and biocompatibility of PDMS when compared to the silicon or nitride silicon-based materials used in traditional microfabrication.

3.3. Signal detection

The signal detection techniques used in surface stress-based biosensors can be broadly classified into optical detection and electrical detection. The two methods can also be further classified into position sensing (photo) detection, fiber interferometry, piezoresistive detection, and capacitive detection.

3.3.1. Optical detection

For the cantilever biosensor, the simplest way to measure the beam deflection resulting from surface stress is the position sensing (photo) detection method (Godin et al., 2003). The basic detection scheme is shown in Fig. 8a, a laser is focused onto the

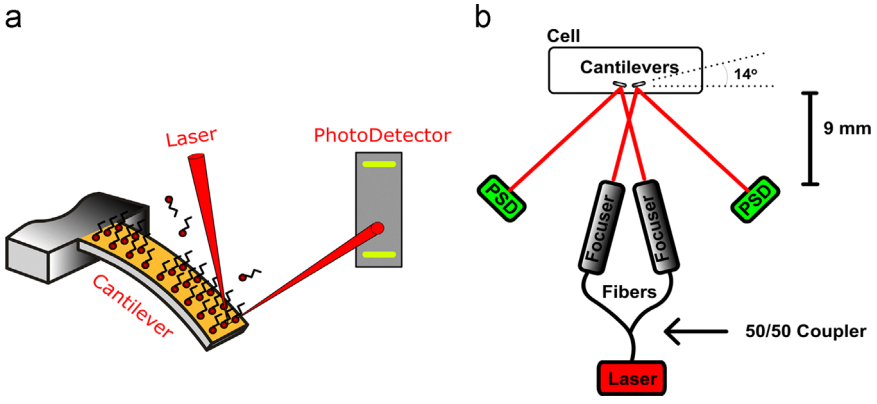

Fig. 8. (a) The optical beam deflection technique is used to monitor the deflection of the cantilever (Godin et al., 2004). (b) Top view of the deflection sensing components. The cantilevers are angled away from each other by 14° in order to accommodate all the components. Image adapted from Godin (2004).

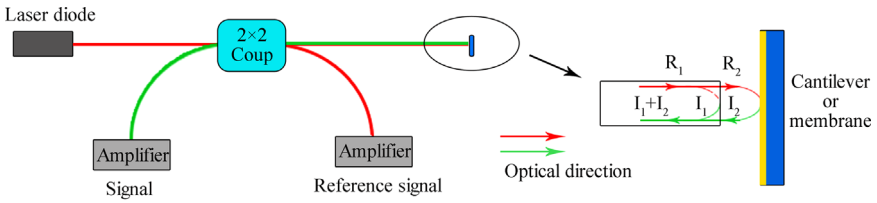

Fig. 9. Schematic diagram of a fiber interferometer which is used to monitor the deflection of the cantilever/membrane. Image adapted from Sang (2011).

end of the cantilever. The information of displacement is monitored using the position sensing (photo) detector (PSD) (Godin et al., 2004). Fig. 8b shows the top view of this deflection sensing components.

As shown in Fig. 8b, the light from a laser diode was coupled to a FC-connectorized single-mode optical fiber. The light is then split into two fibers using a coupler. The two fibers emerging from the coupler were fitted with GRIN (grade d index) lenses to focus the beam onto the cantilevers. The displacement of the reflected laser beams are monitored using two independent position sensitive (photo) detectors (PSD). For the position sensing (photo) detection method, two points must to be noted. First, the single common light source makes it possible to eliminate the effects of laser intensity noise on the differential measurement since both the active and the reference cantilevers experience the same noise. Second, it is necessary that cantilevers were angled away from each other (approximately 10°), because of the geometrical constraints imposed by the focusers and the PSDs. In addition, this angle prevents stray laser light reflecting from the glass (sealing the cell) from hitting the PSDs, thus influencing the deflection measurement. The exact relationship between the cantilever deflection and the PSD measurement can be obtained by using elementary geometric optics and vector analysis (Beaulieu et al., 2007; Álvarez and Tamayo, 2005; Godin, 2004). Based on the similar principle, a SiN*x* based gold coated microcantilever array is realized to quantitatively measure the activity and inhibition of a model protease immobilized on its surface (Raorane et al., 2008a., 2008b. In this system, a charge coupled device (CCD) camera instead of PSD was used as the detecting element by the authors. Moreover, the exposed silicon nitride surface (all of the cantilever surface outside the sensitive area) was passivated with silanated polyethylene glycol to improve sensitivity, which is a good reference for design.

Another optical detection method commonly used to measure the deflection information of cantilever/membrane is the fiber interferometry (Moser et al., 1993; Rugar et al., 1989). The deflection in the range of 10^{-11} m to 10^{-13} m can be measured (Raiteri et al., 2000). Fig. 9 shows the fiber interferometer used to monitor the deflection of cantilever/membrane. In this case, a cleaved fiber is brought in close proximity (~10 μm) to the cantilever/membrane surface. The deflection information can be measured through detecting the interference signal between the reflected light of the fiber cleaved end and of the cantilever/membrane surface. Fiber optic interferometer is a mature technology and has many advantages, such as good performance, low loss, high bandwidth, safety and relatively low cost, which is suitable for biosensors. However, the fiber's fragility and the difficulty of cleaning the fragile fiber make it unsuitable for use in some cantilever/membrane-based chemical sensor (Godin, 2004). Also, fiber interferometry runs into limitations when the cantilever/membrane suffers large deflections (> 3 μm), thus reducing the dynamic range of the cantilever/membrane-based system.

One smart WLI (white-light interferometry)-based biosensor test system was applied by Sang (2010). Fig. 10 illustrates the principle scheme of the smart WLI-based biosensor test system. The author said that the test system has a better spatial resolution (1 nm) and the membrane deflection information can be read nanometre-accurately and directly, but its time resolution is relatively low (~1 min.). Fig. 11 illustrates a test result of the test system for different conditions of *E. coli*, healthily living and dead (Sang and Witte, 2010b). The deflection caused by living *E. coli* was bigger than dead *E. coli*. Hence, Sang (2010) proposed one hypothesis that the deflection of membrane caused by other conditions of *E. coli* (between healthy living and death) would be the interval values; they can be quantized through lots of experiments, which is useful for the medical test and analysis. Sang et al. proved that the smart WLI-based biosensors have high spatial resolution. The membrane deflections of biosensors can be read nanometre-accurately.

3.3.1.1. Advantage and limitation. Optical detection techniques offer a clean, easy and quick way to isolate the electrical contact point from the electrolyte solution. Another main advantage is that all the deflection-sensing components can be kept outside of the enclosed cell environment. It avoids some level of contaminations in the electrochemical.

However, optical detection always has the problem of heat drift. Thermal effects are clearly evident and occur slowly (over a time scale of hours) for the duration of the experiment. In some cases, radiative heating can increase the microcantilevers temperature to 130 ± 20 °C (Doelle et al., 2006). Thermal effects are particularly troublesome for cantilever/membrane sensors where a gold layer is evaporated on one surface, creating a sensitive

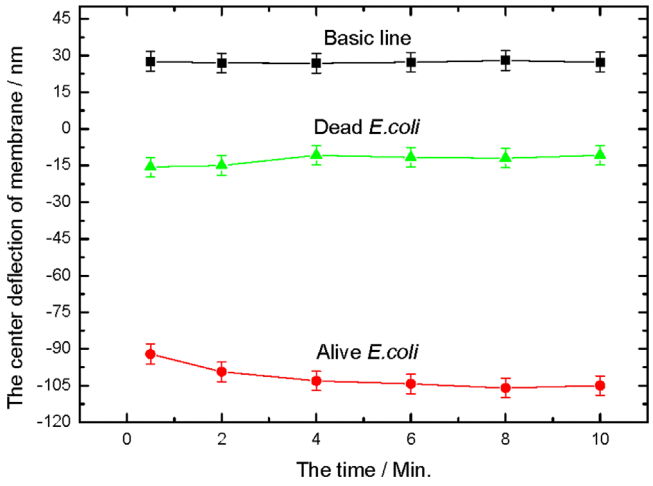

Fig. 11. The center deflection of membrane resulting from water load and different status of *E. coli*. Image from Sang and Witte (2010b).

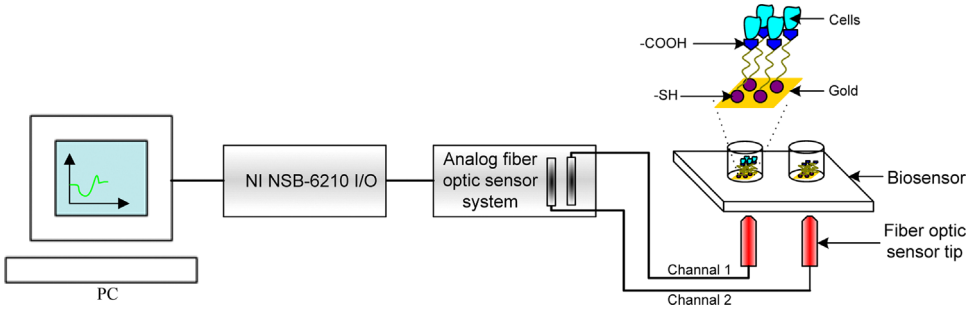

Fig. 10. The principle scheme of the Smart WLI-based biosensor tests system. Image from Sang (2010).

bimetallic. Optical detection systems are still typically large and so they are more suited for bench-top applications than for portable handheld use. Moreover, the optical read-out is technically difficult to scale to large cantilever arrays, which would allow the simultaneous detection of multiple analytes (Raiteri et al., 2001; Von Preissig, 1989). As for the PSDs, they suffer some other limitations. A calibration is needed in order to obtain the actual cantilever deflection from the recorded signal. Ultimately, index of refraction changes of the surrounding medium of the cantilever can produce artificial deflection and the technique cannot be used in opaque media such as blood.

3.3.2. Electrical detection

In order to overcome the problem brought along with the optical detection methods, electrical detection has been proposed.

3.3.2.1. Piezoresistive detection. Piezoresistivity is a commonly used transduction mechanism for MEMS (Barlian et al., 2009), such as force sensors (Duc et al., 2007; Park et al., 2007; Yu et al., 2002; Harley and Kenny, 2000; Hansen and Boisen, 1999; Tortonese et al., 1993), pressure sensors (Cho et al., 2008; Pramanik et al., 2006; Bae et al., 2004; Merlos et al., 2000; Kanda and Yasukawa, 1997), stress sensors (Doelle et al., 2006; Li et al., 2006; Bartholomeyczik et al., 2005), microphones (Bhardwaj et al., 2001), accelerometers (Wang and Xu, 2007; Lee et al., 2003), temperature sensors (Lee and King, 2008; Lee et al., 2008), chemical sensors (Monrudee, 2008; Yang et al., 2003; Hierlemann et al., 2000) and biological sensor.

In the cantilever piezoresistive detection system, a piezoresistive material is embedded near the top surface of the cantilever to form the piezoresistor. In most cases, the location of a piezoresistor has to be close to one surface of the microcantilever to maximize the sensitivity. If the cantilever is stressed by the surface stress, the strain applied to the piezoresistor will cause a change in resistance which can be measured by the electronic methods (Monrudee, 2008). The Wheatstone-Bridge circuit can be used to measure the resistance change (Tortonese et al., 1993). Moreover, the piezoresistors should be protected from the environment by layers, such as silicon oxide and silicon nitride. However, the protection layers can decrease the sensitivity of the sensor system. They should be as thin as possible while still providing sufficient protection. Fig. 12b illustrates the wheatstone-bridge structure (Wen et al., 2013; Harley and Kenny, 2000) which is the typical testing method of cantilever resistance.

Piezoresistive detection technology has been widely studied for surface stress-based biosensors. Marie et al. developed a piezoresistive cantilever system instead of the optical detection method for sensing DNA hybridization (Marie et al., 2002). Research shows that the sensitivity can be improved by increasing length ratio and decreasing thickness ratio of the cantilevers (Yang and Yin, 2007). Many efforts have been continually contributed to modify the structure of piezoresistive cantilever for improving the surface stress sensitivity and stability. A double microcantilever design is developed to improve the measurement sensitivity (Yang et al., 2007). In this structure, the biaxial surface stress in the top immobilized cantilever can be converted into uniaxial strain in the bottom sensing cantilever. The authors also proposed that the sensitivity can be further increased with a higher length ratio and a lower thickness ratio of the two cantilevers. Recently, Yoshikawa et al. developed a membrane-type surface stress-based sensor (MSS) by optimizing the piezoresistive cantilever. The comprehensive structural consists of an "adsorbate membrane" supported by four constrictions called "sensing beams", on which piezoresistors was integrated (Fig. 13). This configuration further improves the sensitivity by a factor of ~4 and it enhanced the thermal stability by self-compensation (Yoshikawa et al., 2012). This structure has a factor of 20 than that of a standard piezoresistive cantilever.

3.3.2.1.1. Advantage and limitation. Compared to the optical detection, piezoresistive detection shows several advantages. It does not have expensive and macroscopic optical components

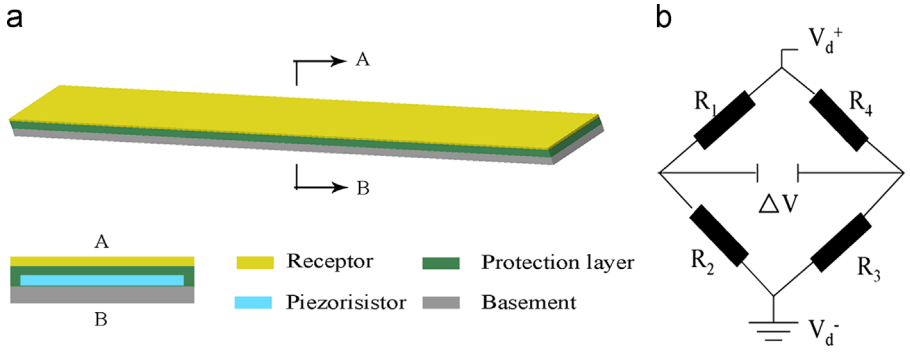

Fig. 12. (a) The structure diagram of piezoresistive cantilever. (b) Wheatstone-bridge structure showing resistors R1–R4. Image (b) adapted from Harley and Kenny (2000).

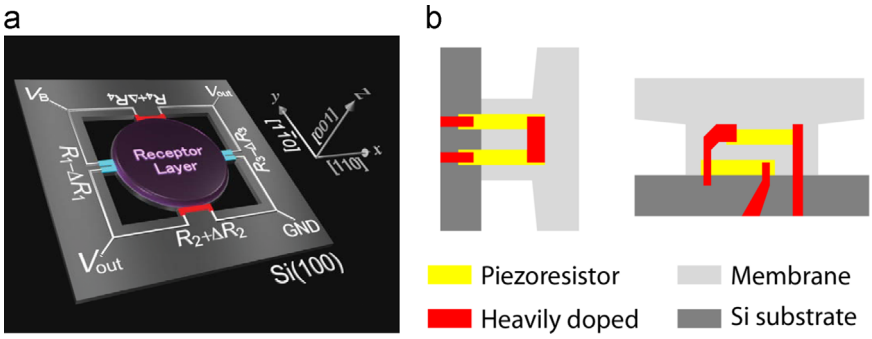

Fig. 13. (a) Schematic illustration of the MSS. The whole surface stress induced on the round center membrane is efficiently detected by piezoresistors (red and blue colored parts) embedded in the constricted beams. (b) The piezoresistors on R1 (left) and R2 (right) in the 2G-MSS chip. Image from Yoshikawa et al. (2012). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and can work in non-transparent solutions. In addition, piezoresistive read-out can be integrated on the same silicon chip supporting the cantilevers/membranes using the same CMOS fabrication technology. It is also well suited for arrays and compact systems, where the sensor is integrated with electronic circuitry, as well as for microfluidic total analysis systems (μTAS) (Tsouti and Chatzandroulis, 2012; Sang and Witte, 2010b; Cha et al., 2008).

Although these piezoresistive cantilevers/membranes have relatively simple electronics structure and do not need extra optical components, their spring constants are typically larger than usual cantilevers/membrane, which thus reduces their sensitivity and is not suited for the surface stress-biosensors because the surface stress is small. In addition, these electronics-based cantilevers have the disadvantage that the deflection sensing electronic structures should be isolated from the chemical environment surrounding the cantilevers. Furthermore, heat can be generated when current passes through the piezoresistor element, which will increases the temperature and lead to thermal drifting. The cantilever/membrane cross-sectional structure is complex because the resistor must be embedded into a dielectric layer to operate in electrolytes; there are consequently limits in fabricating thin, highly sensitive, cantilevers/membrane. Hence better detection methods are studied and developed, such as capacitive detection.

3.3.2.2. Capacitive detection. Capacitive measurement can reveal much information coming from the change of dielectric or displacement. Since Blanc et al. (1996) developed the capacitive sensors, this sensing technique has been demonstrated for detection of protein binding (Rickert et al., 1996), DNA hybridization (Cai et al., 2004), immunoassays (Fernández-Sánchez et al., 2004) and bacterial detection (Yang et al., 2004).

One conceptual design of the micromembrane sensor is illustrated in Fig. 14. When selective chemical/biochemical reactions occur between the target and probe molecules on the sensor surface, the changes in the intermolecular forces will induce a surface stress change which can cause the curvature/deflection of membrane. The change of effective gap between the two electrodes will induce the capacitance alteration. The deflection (curvature change) of membrane is a function of the type of the surface reaction. Thus the information of the probe molecules can be tested through measuring the capacitance change.

In general, the surface stress-based capacitive membrane biosensors have two membranes (Zhang et al., 2013; Sang, 2010; Tsouti et al., 2008; Zimmermann et al., 2008). Fig. 15 illustrates the operational mechanism of the biosensors. One membrane acts as the active membrane and the other as reference. The active membrane is sensitized to react with specific analytes. The analytes will not present on the reference membrane, it only remains sensitive to other environmental factors that can also result in a deflection of the active membrane, such as temperature variations (bimetallic effect), laminar or turbulent flow around the

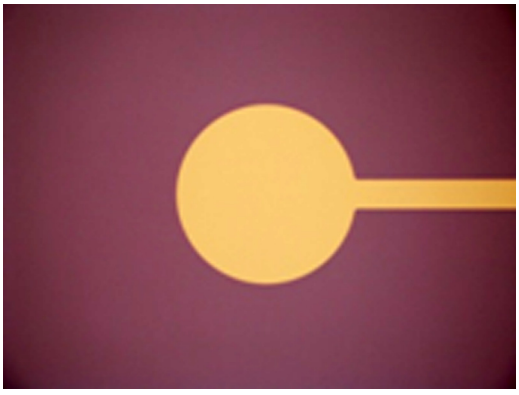

Fig. 16. Surface stress-based circular electrode/membrane biosensor.

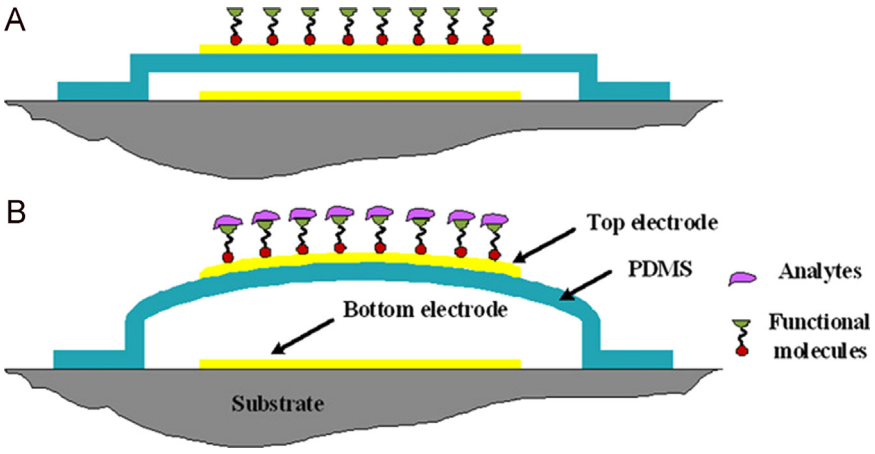

Fig. 14. A is the schematic diagram of surface stress-based capacitive biosensor. B illustrates the deflection of the membrane due to a compressive surface stress change caused by the reaction between the functional molecules and analytes on the sensor surface. Image from Zhang et al. (2013).

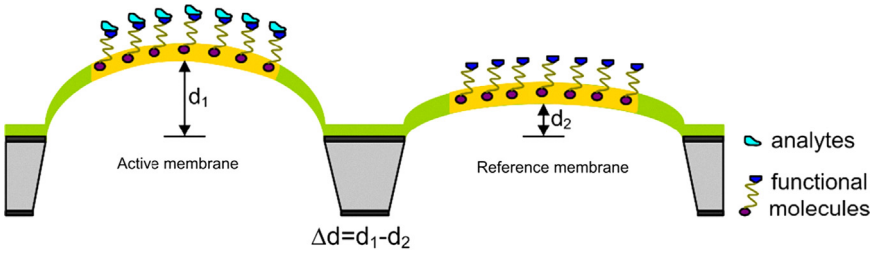

Fig. 15. The operational concept of the PDMS membrane biosensor. Image from Sang and Witte (2010b).

membrane, vibrational (including acoustic) noise, non-specific binding (including the swell induced by solution), etc. The differential capacitive signal is solely due to the interaction of the analytes with the membrane. This design has very sensitive surface stress measurements for analytes detection (Zhang et al., 2013; Sang, 2010; Satyanarayana, 2005).

During our research, the Finite Element Analysis (FEA) is a useful tool for the optimization of the sensor geometry parameters. ANSYS® is a popular software used to analyze the details and properties of these sensors (Zhang et al., 2013). Based on our research (MicroNano System Research Center, Taiyuan University of Technology, China), it was found that the square electrode capacitive biosensors have the boundary effect, which is one main factor to influence the precision of the sensors for the capacitive readout. The circular electrode has smaller fringing effect. Hence, a surface stress-based circular electrode/membrane biosensor has been designed and fabricated, as shown in Fig. 16. The structure was optimized using FEA. It was found that the circular membrane's sensitivity is 20% higher than that of square membrane (Zhang et al., 2013), as shows in Fig. 17a. In addition, the thermal strain mismatch dropped much sharply when the thickness of gold increases, though the sensitivity also dropped slightly (Fig. 17b), so we can use this method to minimize the effect of thermal strain mismatch.

3.3.2.3. Advantage and limitation. Though the surface stress-based membrane capacitive biosensors have some disadvantages, for example, the complexity of the fabrication process, difficulty in realizing the micro-capacitive detection circuit, and the unreliability of membrane, this technique is highly sensitive. The sensing platform can also be scaled up by leveraging the existing knowledge of electronic multiplexing and integrated circuits (Thaysen et al., 2001). The capacitive read-out electronics can be integrated on the same silicon chip supporting the sensor layer using the same CMOS fabrication technology. Moreover, the capacitive read-out is well suited for arrays and compact systems, where the sensor is integrated with electronic circuitry. Furthermore, with the advances in technology the disadvantages can be overcome, so the surface stress-based capacitive membrane biosensor is very promising.

4. Critical remarks

As illustrated above, surface stress-based biosensors provide a promising platform that combines biological/chemical selectivity and high sensitivity while offers the possibility to realize biosensing applications. Tremendous efforts have already been made on surface stress-based biosensor applications. However, a number of key points need to be addressed before realizing the “ultimate application” of surface stress-based biosensors. In this review, we have analyzed the reports of surface stress-based sensors from the sensitive element and materials, to the advantages and limits of detection principles. Most of the reports are related to the biosensor applications in a liquid environment. The sensitivity needed to be enhanced and the ultimate goal of single-molecule sensing need to be accomplished. For the liquid cases, the influence of various parameters such as concentration, diffusion coefficient, velocity of the surface binding reaction, and the reaction kinetics is very different. Hence, extended analytic experiments are needed to demonstrate the respective performance under controlled conditions. For example, most surface stress-based biosensors encounter the problem of low selectivity and reproducibility. As for the cantilever biosensors, another drawback that can not be ignored is slow surface stress changing process when

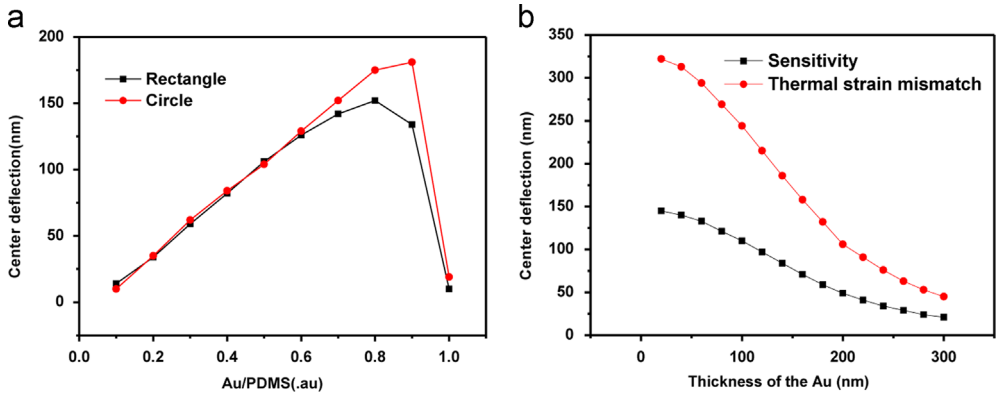

Fig. 17. (a) The sensitivity comparison of square and circular membrane under different coverages. (b) The sensitivity and thermal strain mismatch with different thicknesses of Au. Image from Zhang et al. (2013).

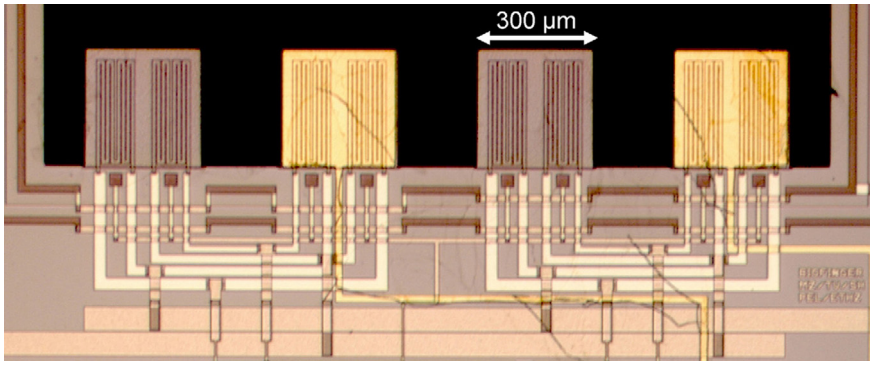

Fig. 18. Sensor array with four microcantilevers. Sensing and reference cantilever are paired (gold-coated sensing cantilever, and uncoated reference cantilever) to achieve a dual-cantilever symmetric Wheatstone bridge configuration. Image from Zimmermann et al. (2008).

used in liquid. It is found that once the cantilever is immersed in liquid, significant detection can be observed (e.g. 0.5 lm over 15 min). Irrespective of whether the cantilever is bimetallic (i.e. the effect is not thermal in origin), such effect occurs and usually takes several hours (up to 10 h) to stabilize. Parameters such as adhesion (i.e. adequately transferring the surface stress in the sensing film to the substrate), surface morphology (grain size, roughness, crystallographic orientation) and cleanliness of the metal film can have both qualitative and quantitative effects on the measured surface stress. It is therefore essential to characterize and study these effects on the sensor response.

The transporter of analyters also affects the signal-to-noise ratio (S/N) of the surface stress-based biosensors. However, the total analyte flux is strongly affected by the sensor size and shape. Till now various approaches have been taken to improve the analyte transmission towards sensors. In addition, the size, as well as the contact parameters, will definitely affect the sensor behavior. But more research still needs to be demonstrated.

In addition, the detection limitation in the micro/nanoscale and possible parameters affects the sensor performances and applications. To enhance the performance, very recently the use of arrays was demonstrated. The most promising feature of the cantilever/membrane-based biological/chemical sensor is the ability to relatively cheaply micro-fabricate cantilevers/membranes in arrays. So it is possible to perform parallel biological/chemical sensing. These array-based sensors have multiple applications in a wide range of industries. Many examples exist in the medical and pharmaceutical industries, including the detection of various diseases by monitoring a patient's blood chemistry or breathing. Lang et al. produced a kind of cantilever array-based sensing as a prototypical artificial nose. The position of the cantilever sensors is read out by the beam deflection technique. This device can be operated in various media such as ambient air, vacuum, and liquids, which allows the characterization of gaseous analytes and liquids in chemistry and biochemistry (Lang et al., 2002, 1999, 1998).

Fig. 18 shows the micrograph of one cantilever array, whose detection method is piezoresistive detection. The sensing cantilevers (2nd and 4th cantilever in the array from the left) have an additional gold layer on top, while the reference cantilevers (1st and 3rd) have only silicon nitride as protective layer on the surface. Sensing and reference cantilevers are paired to achieve a dual-cantilever symmetric Wheatstone bridge configuration. Common-mode signals affecting both cantilevers are directly canceled out by the bridge design. The Wheatstone bridge output features a deflection sensitivity of 0.7 μV/nm per volt bias voltage, and the offset of the Wheatstone bridge is 25 ± 5 mV at 5 V bias voltage (Zimmermann et al., 2008). The cantilever array-based sensors give us a design concept.

Tsouti et al. (2012) presents a capacitive surface stress-based membrane biosensor array, consisting of a total of 256 biosensing elements, as shown in Fig. 19a. The deflection variations of the ultrathin Si membranes are transformed into capacitance changes. The array was proved to be able to distinguish between different species when it was spotted with three different oligonucleotides (CD19, CD17 and CD8). This kind of biosensors have low power consumption and very suitable for portable type devices, for instance, for Point-of-Care diagnostics. But the Si membrane-based biosensor array has low sensitivitity.

It was found that the surface stress-based PDMS membrane biosensors have higher sensitivity than Si membrane biosensors (Sang and Witte, 2009). In the following researches, the authors found that the gold coverage has effect on the membrane deflection (Zhang et al., 2013; Sang and Witte, 2009). The center deflection reaches maximum when the ratio (R_{gold}/R_{PDMS}) is about 0.9 in circular membrane (Zhang et al., 2013). Larger membrane shows higher sensitivity. Based on our research, a surface stress-based PDMS membrane biosensor array has been manufactured by MNSRC (patent protection in China) (Fig. 19b). The verification tests are currently underway.

It is possible to obtain the “signature” of complex molecule and eventually achieve effective molecule identification by collecting the varying response of each sensor in the array. Nevertheless, most of these techniques are still under investigation, and their integration for device fabrication represents a real challenge for the scientific community and the related commercialization of surface stress-based biosensors. There are only few companies sell the surface stress-based biosensors so far. Although some challenges must be conquered to realize the production and industrialization, many researchers have been striving in this area.

5. Summary and prospects

The developments of surface stress-based biosensors have been summarized in this review. Several challenges remain although there are many well-established methods to fabricate the surface stress-based biosensors and to understand their properties. As with many chemical/biological sensors, the sensitivity has increased tremendously in recent years, but not much improvement has been seen in the area of selectivity. The selectivity of a biological/chemical sensor is limited by the properties of the sensor coating material. The sensor coating material also plays a key role in determining the sensitivity and the amount of non-specific binding. Hence, surface coating technology used for biosensor functionalization and passivation needs to be optimized for improving the biosensor performance. But it is not a feasible task to find the specific sensor

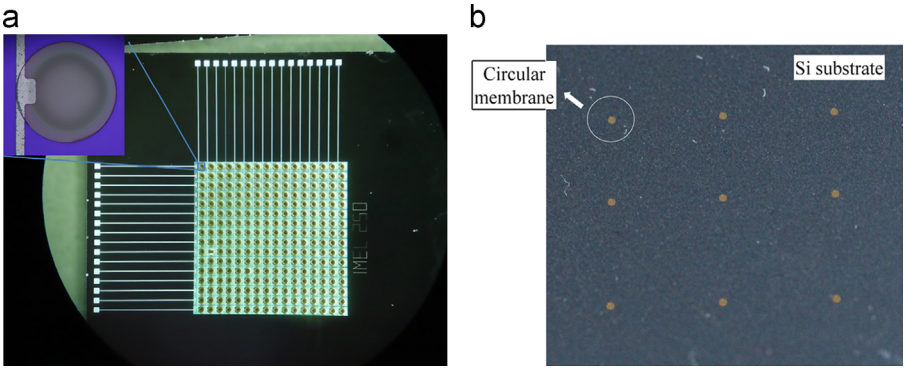

Fig. 19. (a) The surface stress-based capacitive biosensor array designed by Tsouti et al. Image adapted from Tsouti et al. (2012). (b)The photo of surface stress-based capacitive circular membrane biosensor array by MNSRC.

coating materials for the extremely large number of targets. To circumvent this problem, sensor arrays with coatings that interact with many biological/chemical species at different levels have been investigated in order to obtain unique signatures for each of them. The advantages that sensor arrays offer over individual sensors are sensitivity to a wider range of analytes, improved selectivity, simultaneous multi-component analysis, and the capability for analyte recognition rather than mere detection. In addition, the fabrication method should be improved to get more uniform and small surface roughness of sensitive element.

Obviously the surface stress-based biosensors can measure the effects of bio-analytes, and analyze their properties based on the change of sensitive element deflection resulting from the alteration of surface stress. The technique is advantageous since the process does not require any labeling of target molecules or the addition of redox probes. It allows the detection in opaque media. The surface stress-based micro-membrane capacitive biosensor can be made into chip using the CMOS fabrication technology, where the sensor is integrated with electronic circuitry. In addition, the capacitive read-out is well suited for portable type devices and arrays. Furthermore, circular PDMS membrane is a good choice for the surface stress-based capacitive biosensors, which can reduce the fringing effect. The gold covering ratio (R_{gold}/R_{PDMS}) has important effect to the sensitivity of biosensors. The thickness of gold layer on PDMS membrane not only affects the sensor sensitivity, but also affects the thermal strain mismatch. Until now, the surface stress-based circular PDMS micro-membrane biosensors is the best design.

Further, as the utilization of such biosensors grows significantly, the demand for novel and more effective methods for their preparation will certainly increase. Other improvements may be done to make the biosensor better, for example, more portable, more miniaturized. However, we should also pay attention to some potential environmental and health issues when more and more nanostructures are produced in laboratories because it may be have some long-term impacts on environment and health while handling these structures.

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