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A novel PDMS micro membrane biosensor based on the analysis of surface stress

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ABSTRACT

The biological and medical application of biosensors is more and more important with the development of technology and society. Detection of cells and biological molecules utilizing biosensors based on the analysis of surface stress would facilitate inexpensive and high-throughput test and diagnosis. This paper presents a biocompatible surface stress-based polydimethylsiloxane (PDMS) micro membrane biosensor. Each biosensor chip consists of two available PDMS micro membranes, one acts as active membrane and the other as reference. Biosensors were functionalized using different functional materials respectively: MUA (11 Mercapto 1 undecanoicacid), MUO (11 Mercapto 1 undecanol) and DOT (Dodecane thiol). Two biosensor test systems were built based on a white light interferometer and a fiber optic interferometer respectively. Finally, testing experiments using *Escherichia coli* (*E. coli*) were performed based on the biosensor test systems we built. The results of the experiments showed that the MUA is a better functional material to functionalize the biosensor membranes than MUO and DOT for *E. coli* detection, some properties of *E. coli*, such as healthily living and dead status, can be analyzed based on the PDMS micro membrane biosensors.

1. Introduction

Nowadays, biosensors become an attractive researched area with a wide variety of important biomedical applications, ranging from diagnostics of cells, DNA (Duggan et al., 1999) and protein micro-arrays, to novel materials for biosensors, tissue engineering, surface modification, systems for drug delivery, and so on. Surface stress-based biosensors as a relatively new class of biosensors have been investigated extensively in recent years. These biosensors use the free energy change, the underlying concept in any binding reaction, and hence offer a universal platform for biological and chemical sensing. The value range of surface stress in such reactions was reported to be 5–50 mJ/m^2 (Widge et al., 2007; Fritz et al., 2000; Wu et al., 2001a,b) or as high as 200 mJ/m^2 (Berger et al., 1997) and 900 mJ/m^2 (Marie et al., 2002). Biological sensing of numerous analytes resulting from surface stress can be achieved using cantilever or membrane as the sensitive element of sensors. When the analytes of interest interact with the sensitized surface, a surface stress is induced, and the cantilever or membrane bends due to the different surface stresses acting on both sides. The sensitivity of sensor to a specific analytes is determined by the chemical functionalization of the sensitized surface of biosensors. Very specific surface functionalizations can be achieved using molecular self-assembled monolayers (SAMs) as sensing layers assembled on the surface of the sensitive part of biosensors. The SAMs can be formed on a gold-coated cantilever or membrane to improve biocompatibility (Widge et al., 2007) and provide sufficient bending stiffness (Yue et al., 2004). Some researchers have demonstrated the capability of surface stress sensors in detecting a variety of reactions using micro cantilever sensors, which include DNA hybridization (Fritz et al., 2000; Marie et al., 2002; Wu et al., 2001a,b; Hansen et al., 2001) and antigen–antibody binding (Wu et al., 2001a,b). However, the cantilever geometry is not the best for sensing in liquid media, because the entire cantilever is immersed in the fluid and non-specific adsorption on the back side of the cantilever could drastically reduce the signal-to-noise ratio (SNR) (Yue, 2004).

Test techniques of biological signal can be broadly classified into label-free and label-based techniques. Label-based techniques rely on the specific properties of labels like fluorescence, chemiluminescence, etc. for detecting a particular target. However, the process of labeling and purification processes is associated with sample losses, which is critical when sample quantity is limited. Labeling processes may also have a detrimental effect on the functionality and stability of molecules like proteins. Mass spectrometry, surface plasmon resonance (SPR) and other optical method are label-free techniques, and they can conquer these disadvantages. Interferometry as one kind of optical method is an important investigative technique in the fields of astronomy, fiber optics, engineering metrology, optical metrology, oceanography, seismology, quantum mechanics, nuclear and particle physics, plasma physics, and remote sensing (Bunch and Hellemans, 2004). A deflection in the

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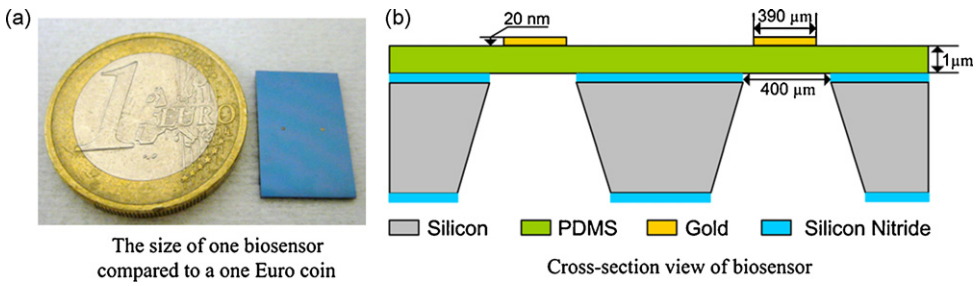

Fig. 1. (a and b) The structure of biosensor.

range of 10^{-11} to 10^{-13} m can be possibly measured theoretically using interferometry.

In this paper, we report on a novel surface stress-based PDMS micro membrane biosensor and its application on *E. coli* detection. Two biosensor test systems were built with a white light interferometer and a fiber optic interferometer, respectively.

2. Biosensor

The PDMS micro membrane biosensors have been successfully fabricated through conquering many challenges, e.g., integration of PDMS processing with conventional microfabrication processes and the fabrication of perfect PDMS thin film. The detailed fabrication procedure has been discussed in our publication "Fabrication of a Surface Stress-based PDMS Micro-membrane Biosensor" (Sang and Witte, in press). The biosensor is composed of two layers: PDMS thin film layer with partly gold coated on its surface and silicon substrate layer, as shown in Fig. 1. The sensitive element — membrane is made of the PDMS thin film, partly gold coated on its surface, and the trapezoidal structure in the silicon substrate. PDMS is the sub material of membrane due to its lower stiffness than silicon, aluminium nitride, silicon nitride and polymethylmethacrylate (PMMA). The Young's modulus of bulk PDMS usually fall within 12 kPa to 2.5 MPa (Tan et al., 2003; Brown et al., 2005; Armani1 et al., 1999; Eddington et al., 2003; Gray et al., 2003), depending on the processing conditions. Furthermore, PDMS has the characteristic of biocompatibility and ease of processing (Tan et al., 2003; Peterson et al., 2005; Ostuni et al., 2000; DeSilva et al., 2006; du Roure et al., 2005). One biosensor chip consists of two available micro membranes, one acts as the active membrane and the other as a reference. The active membrane is sensitized to react with specific analytes. Selective biochemical reactions occurring between the analytes and the micro membrane will induce a surface stress change that causes the membrane to deflect. No analytes present on the reference membrane, it is only used to detect other environmental factors that may also result in a deflection of the active membrane, such as temperature variations (bimetallic effect), laminar or turbulent flow around the membrane, vibrational (including acoustic) noise, non-specific binding, etc. The differential signal obtained by subtracting the reference signal from the active membrane's response is solely due to the interaction of the analytes with the active membrane. This design has very sensitive surface stress measurements associated with specific analytes interactions on the active membrane's surface, which is one major novelty of the biosensor.

3. Experimental

3.1. Material

Three alkanethiol molecules with different functional end groups: MUA: SH–$(CH_2)_{10}$–**COOH**, MUO: SH–$(CH_2)_{11}$–**OH** and DOT: SH–$(CH_2)_{11}$–**CH_3** were used as the biosensor membrane coating layers. The functional materials were first deployed standard solution (concentration: 0.1 mM). *E. coli* (Institute of Physics, TU-Ilmenau, Germany) was selected as the analytes. The *E. coli* was first cultivated in a LB medium for 24 h at 37 °C under stirring. Afterwards the *E. coli* sample solution with the concentration of 1.7×10^3 cells/µl was produced.

3.2. Experimental arrangement

For the measurements, two biosensor test systems were built. One is with a fiber optic interferometer as shown in Fig. 2(a) (FOI-based biosensor test system), where the membrane deflection signal is converted to electric signal (voltage) through the fiber optic interferometer system (TETRA®, TETRA Gesellschaft für Sensorik, Robotik und Automation mbH, Ilmenau Germany) and monitored by the computer using software. NI USB-6210 I/O (NATIONAL INSTRUMENTS®, National Instruments Corporation) is the adapter between computer and interferometer system. The FOI-based biosensor test system has a higher time resolution (<0.5 s) and can monitor signals continuously, but its spatial resolution is relatively low (min. 20 nm) because it is affected easily by the topography of membrane and the distance between fiber sensor tip and membrane. The other test system is with a white light interferometer composed of light source, camera system and piezoelectric system, as shown in Fig. 2(b) (smartWLI-based biosensor test system, smartWLI: Gesellschaft für Bild- und Signalverarbeitung (GBS) mbH, Ilmenau Germany). The whole system is controlled by a

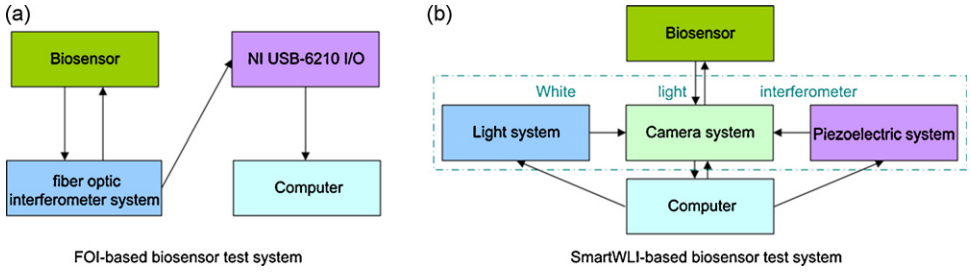

Fig. 2. (a and b) The principle schemes of the two biosensor test systems.

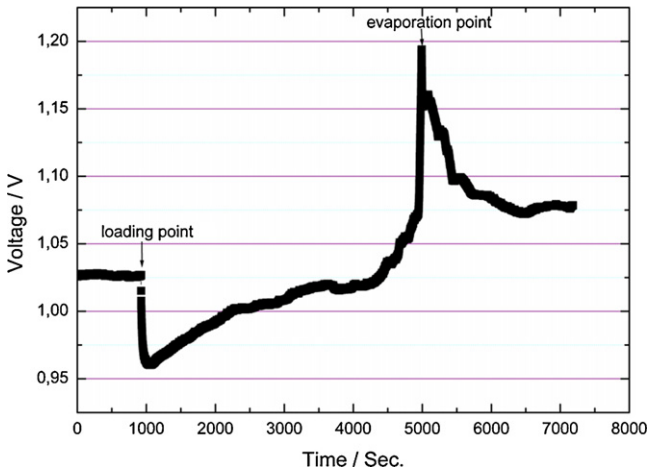

Fig. 3. The center deflection of biosensor membrane during the functional process.

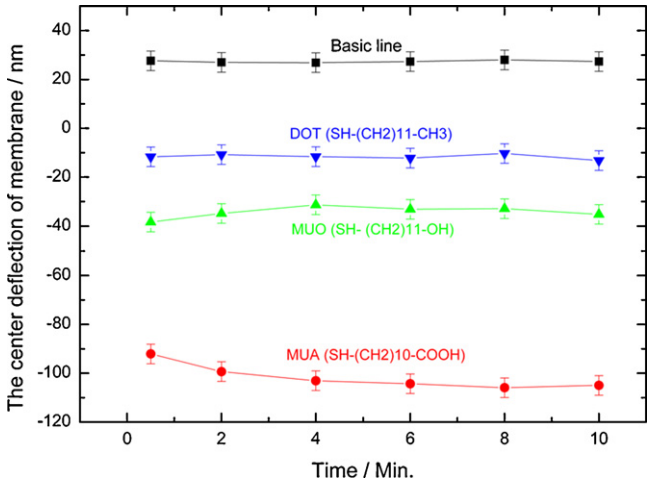

Fig. 4. The center deflection of different functional membranes due to the surface stress resulting from the bond with *E. coli.*

computer. The smartWLI-based biosensor test system has a better spatial resolution (1 nm) and the membrane deflection information can be read nanometre-accurately, but its time resolution is relatively low (~1 min).

3.3. Functionalization

Before functionalization, the small solution reservoirs surrounding the micro membranes were created using a biochemical plastic pipe with ~4 mm diameter and bonded together with the biosensors using the uncured PDMS method (Sang and Witte, in press). Three standard functional solutions (20 μl, 0.1 mM) were then loaded into different biosensor reservoirs using micropipette. The membranes were incubated in thiol solutions for the coating formation of SAMs until evaporation of all solutions. The functionalization process can be detected using the FOI-based biosensor test system due to its continuity, as shown in Fig. 3. Once the membranes were functionalized, the entire chips were washed in ethanol to remove the non-specifically bound thiol molecules.

3.4. Selection of SAMs for E. coli detection

The smartWLI-based biosensor test system was applied in the experiment due to its high spatial resolution. The membrane deflections can be read nanometre-accurately and distinguished obviously. First, 35 μl distilled water was loaded on the membranes to measure the basic deflection signals for normalizing three different functionalized biosensors by MUA, MUO and DOT. After the recovery of membranes, 35 μl *E. coli* medium (1.7×10^3 cells/μl) was loaded separately to measure the deflection caused by the possible surface stress between *E. coli* and different membranes. Fig. 4 shows the deflection information of the three biosensors.

3.5. E. coli detection based on the smartWLI-based biosensor test system

35 μl distilled water was firstly loaded in the solution reservoirs of the biosensors to measure the basic deflection signals. After the recovery of membranes, 35 μl medium with healthily living and with dead *E. coli* (1.7×10^3 cells/μl) was loaded respectively to measure the deflection caused by the possible surface stress between *E. coli* and membranes. Fig. 5 illustrates the results.

3.6. E. coli detection based on the FOI-based biosensor test system

In the experiment, the membrane deflection signals coming from healthily living *E. coli* and dead *E. coli* were monitored by the FOI-based biosensor test system. First, 20 μl pure medium (no *E. coli*) was loaded to measure the basic reference signals, as shown by the line 1 and 2 in Fig. 6. After the recovery of membranes, 20 μl medium with healthily living and with dead *E. coli* (1.7×10^3 cells/μl) was added respectively to measure the deflection signals caused by the possible surface stress, line 3 and 4 in Fig. 6.

4. Results and discussion

Fig. 3 shows the deflection characteristics of the membranes during the functionalization process. At the first stage, the weight of functional solution was the main factor for the membrane deflection, which made the membrane deflect in $-Z$ direction. The voltage also became small as a result of the reflex of functional solution to laser. With the evaporation of functional solution, the surface stress resulting from the bond between gold and thiol group became the main influence factor. The surface stress to form a strong covalent bond between these sulphur atoms with gold surface atoms is around 185 kJ/mol (Kondoh et al., 1999), hence the voltage became larger during the later stage until one stable level. It can also be

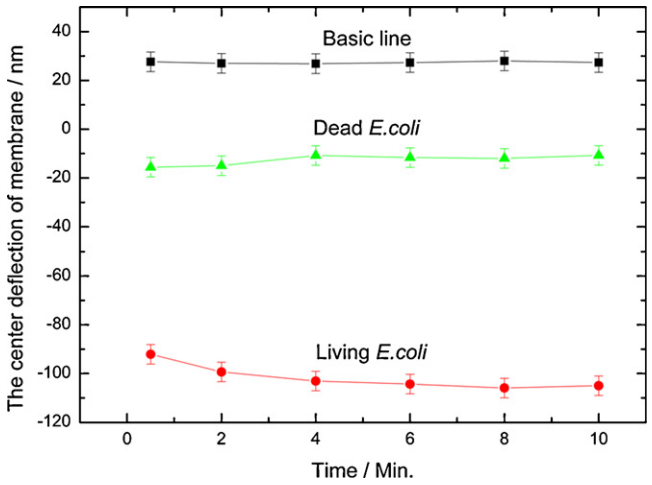

Fig. 5. The center deflection of membrane caused by the different status of *E. coli* compared with by water.

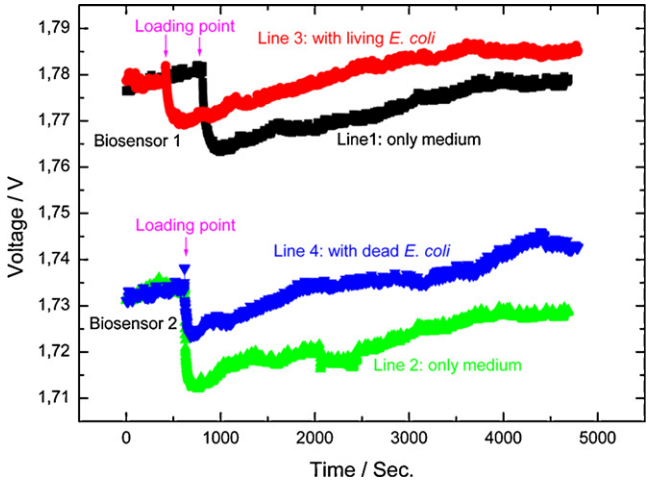

Fig. 6. The comparative signals between only medium and the medium containing living or dead *E. coli*.

concluded that the direction of membrane deflection caused by the weight of solution was opposite to the one caused by the surface stress, which is consistent with the theoretical prediction.

Fig. 4 shows the deflection information when *E. coli* located on three different membranes functionalized by MUA, MUO and DOT respectively. The surface stress presented between *E. coli* and the membranes, and the direction of membrane deflection induced by the surface stress was opposite to the one induced by the weight of medium. The variations of membrane deflection were small during the whole test period (10 min), since no big change of the medium and the absorption quantity of *E. coli* on the membranes was observed for a short period. However the values of surface stress differ for different functional membranes. It can be explained by the theory of cell biology and biochemical molecular reaction. Phospholipids and amino acid are the main chemical structure of out layer component of cell. =O is one main chemical bond of phospholipids (http://en.wikipedia.org/wiki/Biochemistry). An amino acid is a molecule that contains two functional groups, an amine ($-NH_2$) and a carboxylic acid (-COOH) (http://en.wikipedia.org/wiki/Biochemistry). Amino acids are categorized into three groups based on the nature of the side chains. Nine of the amino acids have side chains that are nonpolar. Almost 50 percent of the amino acids that are present in proteins have nonpolar side chains. The second category of amino acid contains six different molecules that have polar side chains. Finally, a group of five amino acids have side chains that are not only polar, but charged. In addition, *E. coli* is a Gram negative bacterium, and there is lipopolysaccharide (LPS) embedded in the outer leaflet of the outer membrane. LPS is composed of three distinct components: lipid A, oligosaccharide core, and O-antigen, which includes a large amount of –COOH, –OH, –H and =O chemical bond (Feulner, 2003).

Some possible bonds between *E. coli* and different functionalized membranes could happen based on the chemical molecular bonds and reactions: (1) MUA: functional end group –COOH has the possible interaction with $-NH_2$, –COOH, –OH and =O and bond together, which induced the surface stress resulting in the deflection of the membrane. In addition, electrostatic forces and the Van der Waals force between molecules may also play roles on the membrane. (2) MUO: only the chemical bond =O may have the interaction with functional end group –OH and bond together. Furthermore, the Van der Waals force may be an acting force. All these factors resulted in the surface stress and accordingly the membrane deflection. (3) DOT: the main connections between the functionalized membrane and *E. coli* were achieved by the van der Walls force, but van der Waals force is relatively weak compared to other chemical bonds, which is normally smaller than 5 kJ/mol. In conclusion, the possible amount of *E. coli* attached on different functionalized membranes is: –COOH > –OH > $-CH_3$. Similar conclusion has also be reported in the reference (Margel et al., 1993), –OH > $-CH_3$ for peptide, protein, and cellular interactions with self-assembled monolayer model surfaces.

Furthermore, the strength of surface stress (interaction stress or chemical bond stress) is also the main factor to affect the membrane deflection. The strength of bonds varies considerably; there are "strong bonds" such as covalent or ionic bonds and "weak bonds" such as dipole–dipole interactions, the London dispersion force and hydrogen bonding. In general, strong chemical bonding is associated with the sharing or transfer of electrons between the participating atoms. Bonds vary widely in their strength which is associated both with the energy required to break them, and the forces they exert on the atoms they hold together (http://en.wikipedia.org/wiki/Chemical_bond). Therefore, the surface stress strength between alive *E. coli* and different functionalized membranes is –COOH > –OH > $-CH_3$, which is consistent with the experimental result shown in Fig. 4 and MUA is the best functionalized material for *E. coli* detection.

Fig. 5 illustrates the test results for different conditions of *E. coli*, healthily living and dead, based on the smartWLI-based biosensor test system. The deflection caused by living *E. coli* was bigger than dead *E. coli*. The hypothetical reason is that the surface stress resulting from living *E. coli* is bigger than dead *E. coli*, some changes on the membrane of *E. coli* will happen after it dies, especially for the outmost lipopolysaccharides (LPS). LPS is a major component of the outer membrane of *E. coli*, contributing greatly to the structural integrity of the bacteria, and protecting the membrane from certain kinds of chemical attack. LPS also increases the negative charge of the cell membrane and helps to stabilize the overall membrane structure. It may induce the death of the *E. coli* if LPS is mutated or removed. For example, the LPS collapsed after death by heating, and different folding of the Braun protein resulted in a decrease of the periplasmic space, increased the cytoplasmic turgor pressure due to water afflux in the most concentrated compartment (Cerf et al., 2009). These changes reduced the bond way and the amount of bonds between *E. coli* and biosensor membrane, and thus the deflection became smaller. One hypothesis can be proposed 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.

Fig. 6 shows the comparison of active signals and reference signals obtained from FOI-based biosensor test system. Lines 1 and 2 represent the basic reference signals when 20 μl pure medium (no *E. coli*) was loaded on the membranes. The voltage had a big change at the loading point because the membrane deflections were first mainly caused by the medium weight; in addition, the reflex of medium to laser was another reason to make the test voltage become small. With the evaporation of medium, the influence factors of weight and reflex became smaller and the signals increased again. The active signals shown by lines 3 and 4 were achieved when 20 μl medium with living and with dead *E. coli* (1.7×10^3 cells/μl) was loaded into the reservoirs, respectively. The primary deflections also came from the weight and the reflex of *E. coli* medium, so at the loading point the active signals became smaller as well. However, the voltages of active signals were bigger than the reference signals. The reason is the surface stress resulted from the bonds of *E. coli* with the functionalized membranes, and the direction of membrane deflection caused by the surface stress was converse to by the weight of medium, which was consistent with the theoretical analysis. The active signals increased with the evaporation of medium. The last voltage may be bigger than the origination point

due to the lasting surface stress induced by the long time physical absorption of *E. coli* on the membrane surface. However, the difference of signals between living *E. coli* and dead *E. coli* is not so obvious as the results from the smartWLI-based biosensor test system. The reason is the spatial resolution of the current FOI-based biosensor test system is low, which can be improved by using the better FOI system.

5. Conclusions

The novel PDMS micro membrane biosensor based on the analysis of surface stress is successful and has good biocompatibility. The proposed novel scheme by applying two available membranes increases the sensitivity of biosensors. Two biosensor test systems have been built based on the white light interferometer and the fiber optic interferometer respectively. The smartWLI-based biosensor test system has a high spatial resolution (1 nm) and can read deflection signal nanometre-accurately and directly. The FOI-based biosensor test system has a better time resolution (<0.5 s) and is able to monitor the deflection signal continuously. For *E. coli* detection, our experimental results show that the MUA is a better functional material than MUO and DOT to functionalize the membranes. The conditions of *E. coli* can be detected based on the deflection tests. For the biological and medical applications, other kinds of cells or molecules can also be detected based on the biosensor test systems by functionalizing the membranes with different functional materials. The analysis of some pathological changes of cells or molecules can be carried out based on the alteration of membrane deflection. The successful work also promotes our other PDMS biosensor design and realization — Concept of a Microfluidics and Tunneling Effect-based BioMEMS to Detect Cells (Sang et al., 2009).

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