Biosensors and Bioelectronics 130 (2019) 420–426

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Gentamicin drug monitoring for peritonitis patients by using a CMOS-BioMEMS-based microcantilever sensor

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ARTICLE INFO

Keywords:
Microcantilever
Gentamicin
Electric field
Miniaturization sample
Low-cost package

ABSTRACT

We developed a Complementary Metal-Oxide-Semiconductor Bio-Microelectromechanical Systems (CMOS-BioMEMS) based piezoresistive microcantilever sensor for detecting gentamicin, a peritonitis therapeutic small-molecule drug. In recent years, the patient-centric concept has been emphasized. In such a trend, therapeutic drug monitoring (TDM) is especially crucial for patients with peritonitis to avoid adverse reactions from a high concentration of gentamicin in the blood. With the aid of a commercialized semiconductor manufacturing process, the microcantilever sensing platform can serve as a portable, low-cost device and offer real-time detection. With chemical surface modification and capture antibody immobilization, the sensor can detect the small-molecule (< 2 kDa) gentamicin directly. We also modified the pH value of the buffer solution and applied an external electric field to promote sensor sensitivity. Comparing the change of the signals in a non-electric field of antibody immobilization and a 60-volt electric field of antibody immobilization showed that the average signal response increased 1.8 times. In the detection of gentamicin with different concentrations of 10–200 μg/mL, the limit of detection (LOD) of the sensor was 9.44 μg/mL. Finally, the detecting result of a microrcantilever sensor was compared with the one measured by a common instrument in hospital, and the high correlation was expressed between them in gentamicin detection. The CMOS-BioMEMS-based piezoresistive microcantilever sensor has been demonstrated to have great potential as a point-of-care (POC) device for real-time drug concentration monitoring.

1. Introduction

With the advent of a society characterized by an aging population, the need for healthcare is rising and the concept of a patient-centered medical environment is becoming more important. Among several clinical issues pertaining to patient care, attention has been paid to adverse reactions to drugs. To avoid the patient suffering from adverse reactions, the doctor implements therapeutic drug monitoring (TDM) to prevent drug toxicity from overdose (Sirot et al., 2006). Through TDM, medical staffs can monitor the drug level in the blood and adjust the effective drug dosages, and then maintain the active treatment drug's appropriate concentration in the blood.

Patients suffering from renal disease require peritoneal dialysis (PD), a kind of purifying blood treatment. However, during treatment, a common, dangerous disease called peritonitis may be caused by bacterial infection, chemical substances and physical damage. Peritonitis, an inflammation of the parietal or visceral peritoneum, may result from bacterial infection when the patient replaces the dialysis fluid without paying attention to personal hygiene. A patient suffering from infection by gram-negative, the most common culture in peritonitis, needs to receive the gentamicin medicine, the most commonly used in aminoglycoside to heal infection by gram-negative. While gentamicin is able to inhibit gram-negative culture growth, it has to be controlled with a specific concentration in the blood. Otherwise, the therapy may be inefficacious under a low concentration or cause irreversible toxicity adverse reactions in the patient, for example, kidney damage, ear disorders and nerve damage (Lacy et al., 2001–2002; Micromedex, 2003).

Gentamicin, a widely used antibiotic drug for patients with peritonitis, is given as a once a day dose (OD) at a concentration between 16 and 24 μg/mL in the blood (Gilbert and Eliopoulos, 2002; Micromedex, 2003; Murphy, 2001). To monitor the therapeutic drug gentamicin, two traditional detection methods are the particle-enhanced turbidimetric inhibition immunoassay (PETINIA) and the competition-based high-throughput fluorescence polarization immunoassay (FPIA) (Opheim et al., 1984). Nevertheless, those techniques consume large medical resources and require the use of skilled technicians, milliliter-scale

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https://doi.org/10.1016/j.bios.2018.09.014
Received 29 June 2018; Received in revised form 23 August 2018; Accepted 3 September 2018
Available online 07 September 2018
0956-5663/

sample volumes and long turnaround times (Fritz et al., 2000).

Several modern biosensing technologies, such as the field effect transistor (FET) (Wang et al., 2005), surface plasmon resonance (SPR) (Lee et al., 1990), quartz crystal microbalance (QCM) (Cooper and Singleton, 2007), and the microcantilever (MCL) biosensor (Berger et al., 1997; Datskos and Sauers, 1999; Guanghua et al., 2001; Lavrik et al., 2004; Wee et al., 2005; Zhang et al., 2004), have been developed for molecule detection. Among them, the MCL biosensor is the only label-free method that can measure the small molecules of a molecular weight (MW) less than 2 kDa (Berger et al., 1997). In addition, because the piezoresistive MCL biosensor utilizes an embedded piezo-resistor to gain the resistance-change signal of the deflection induced by the molecular recognizing generated surface stress of the MCL, the readout system of the sensor can be extremely miniaturized. Because of this benefit, several groups developed CMOS based MCL sensors for detecting biomolecules (Arya et al., 2015; Huang et al., 2013; Yang et al., 2008). Some of their MCL sensor chips are manufactured in research laboratories using general micromachining processes that do not provide high throughput and high quality devices (Yang et al., 2008). Although some are fabricated by using 0.35 μm 2P4M CMOS process (Huang et al., 2013), they only can operate the sensor in an environment without the ionic buffer solution. Besides, Yang et al. employed a parallel-cantilever-design based sensor which consisted of an active cantilever, a reference cantilever, and two fixed resistors (Yang et al., 2008). This method of traditional dual microcantilevers with the Wheatstone bridge circuit configurations may achieve improvement in the adjustment of the offset voltage and compensation of temperature drift for sensitive and accurate measurements, but can lead to unexpected or irreproducible results in a biochemical environment (Yen et al., 2013).

Consequently, in this work, we developed a gentamicin detection platform by using the CMOS bioMEMS MCL biosensor. We designed the morphology and size of the MCLs and then employed the commercially standard manufacturing process operated by Taiwan Semiconductor Manufacturing Company (TSMC) foundry for fabricating the sensing chip. This stable and precise fabrication technology not only has a high yield (> 90%) but it also can benefit the MCL biosensor by being highly compatible with integrated circuits, mass productive and system portable, characteristics that make it suitable for TDM demand. Since we operated the MCL sensor in the single free-standing detection mode to avoid the unexpected chemical disturbances for biochemical measurement (Yen et al., 2013), the sensor needed to be integrated with the strategy for real-time, eliminating the temperature coefficient of resistance (TCR) and the bimorph effect (Ku et al., 2018). This method not only improves the bio-recognition signal of the sensor but also reduces the overall detection system. In order to increase the sensitivity of the sensor for detecting gentamicin, we applied additionally an external electric field to better the antibody immobilization on the surface of the MCL (Neels et al., 2004; Yen et al., 2009). The sensitivity was also improved by pH value modification in buffer solution which manipulates the molecular electrical property for intermolecular attraction enhancement. Finally, the MCL sensor was operated in liquid environment without a microchannel device for small amount sample detection and decreasing interference from the flow field and bubbles.

2. Materials and method

2.1. Device fabrication and detection setup integration

The MCL sensing chip is made by the CMOS-MEMS TSMC 0.35 μm Mixed-Signal 2P4M Polycide 3.3/5 V standard manufacturing process. The size is 1.5 mm × 1.5 mm after the manufacturing process, as shown in Fig. 1(a). Multilayer materials in 2P4M are two polysilicon layers and four aluminum layers, respectively. The structure of the MCL is 2P2M. The dimensions of the MCL are 3.5 μm thick, 43 μm wide and 200 μm long to achieve high sensitivity and satisfy the standard process limitation. The SEM image of the chip configuration was shown in Fig. 1(b). There were 6 MCLs for gentamicin detection. In addition, an on-chip fixed piezoresistor, the same as the piezoresistor in the MCL, is for thermal effect compensation; there is also an on-chip aluminum metal sensor for temperature detection. The MCL chip was wire bonded to a Printed circuit board (PCB) chip carrier (20 × 40 mm) for signal readout. Exposed contacts were protected and insulated from the liquid by using DOW CORNING ® 3140 colloid. For further protection, the parylene C coating had been applied to prevent the device from a chemical reaction with acid, alkaline or organic solution during the surface modification or measurement process.

The experimental method using a microchannel system to flow analyzes the chip-sensing area led to a time-consuming extra pumping system. Additionally, bubbles could be induced in the microchannel during flow, thereby possibly resulting in the surface tension of the liquid causing unrecoverable deflection of the MCL and leading to the unrecognized signals. Therefore, we made a specific poly-dimethylsiloxane (PDMS) vessel in the volume of 1.2 mL to process the surface functionalization and antibody immobilization in solution. The vessel was designed for placing indium tin oxide (ITO) conductive glass in order to apply the electric field for intermolecular attraction enhancement as shown in Figs. 1(c) and (d).

2.2. Mechanical property and thermal effect elimination

Several important properties for the MCL device are the gauge factor, the resonance frequency, the stiffness coefficient and the thermal effect. The gauge factor is an index that indicates the relation between mechanical property and electrical property. According to the MCL structure and material parameters, the neutral axis, which is calculated from the bottom of the MCL, is 1.175 (μm), where the piezoresistance position is 0.692 (μm) from the bottom of the MCL. We can determine the gauge factor G = 17.711 as the result of the parameter and the material property experiment. The resonance frequency is an index that indicates whether the structure may have resonance in detection frequency. We measure resonance frequency by the Laser Doppler Vibrometer, resulting in a resonance frequency of 69 kHz. Due to the high resonance frequency (> 5 kHz) of the MCL, we do not have to consider the low frequency from surrounding noise. According to the resonance frequency of the MCL, the spring constant can be calculated as 3.598 N/m to determine whether the MCL is strong.

The multilayer MCLs are sensitive to temperature variations in the environment surrounding the sensor. Therefore, the thermal-induced signal, including the temperature coefficient of resistance (TCR) and the material bimorph effect, becomes a major issue for the molecular detection of the MCL biosensor. To eliminate those thermal effects on the sensor, we introduced a strategy that included employing a fixed aluminum on-chip temperature sensor (see Fig. 2(d)) and an embedded piezoresistor in the MCL as a self-sensing temperature transducer. Through temperature-gradient measurement set by a 10TEC-150 controller between 24 °C and 28 °C (Fig. 2(a) and (b)), the relationship between these two resistances and the temperature was expressed as two quadratic functions as listed in (1) and (2). In the functions where R_{Metal} was the resistance change of the aluminum temperature sensor, $R_{\mu-cantilever}$ was the resistance change of the microcantilever sensor and *a-f* were related constants of functions. We could then get the current temperature through the function (1) by measuring the resistance change of the on-chip metal temperature sensor. Once the temperature was measured, we substituted the temperature into the function (2) and then obtained the resistance change of the MCL induced by TCR and the bimorph effect. This thermal effect compensation can be operated through mathematical processing by the LabVIEW program without any additional instrument or device:

$$R_{Metal} = a(T - T_0)^2 + b(T - T_0) + c \quad (1)$$

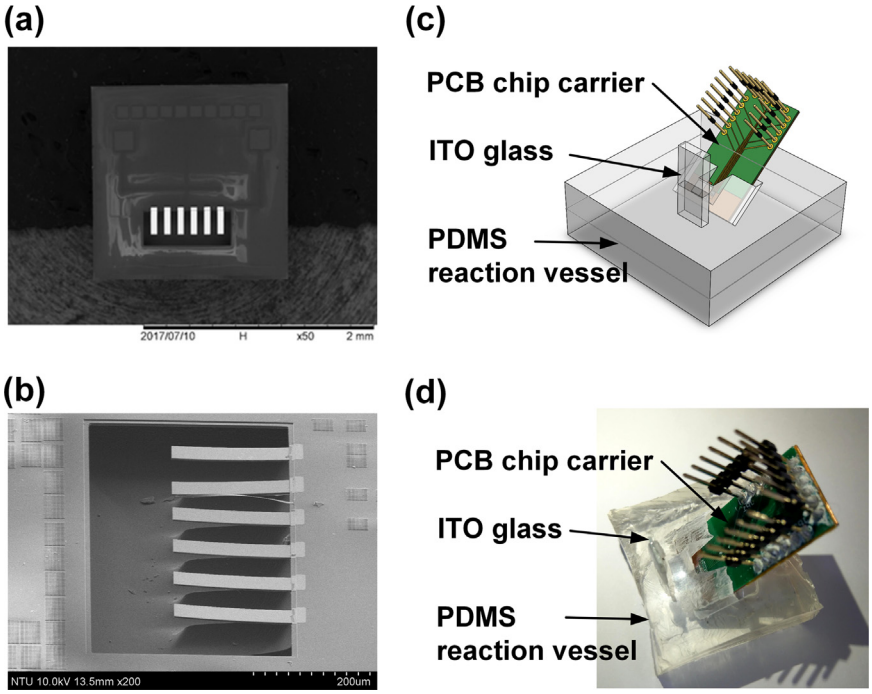

Fig. 1. (a) The SEM top view of the MCL with 6 MCLs in each die. (b) The SEM side view of MCL deformation via residual stress release after the isotropic dry etching process. (c) The schematic of immobilizing the modification process for linker and antibody immobilization in the PDMS reaction vessel. (d) The entity experiment diagram illustrating that the MCL is made by a specific package, and the reaction vessel is made by PDMS molding techniques that make PCB chip carrier and ITO glass a tight fit with PDMS.

$$R_{\mu-cantilever} = d(T - T_0)^2 + e(T - T_0) + f \quad (2)$$

As the MCL resistance floated by the temperature variation in the surroundings, the floating signal could be compensated by the current resistance minus the estimated resistance in the specific temperature which was the established function. In addition, the response signal of the chemical reaction from the antigen-antibody was much lower than 100 mΩ in low-concentration detection. Hence, the MCL device could have the smaller blank signal after thermal compensation, and the MCL device could achieve the better response signal and the lower concentration detection. As shown in Fig. 2(c), the response signal of the resistance was floating before thermal compensation. After thermal compensation was applied to the current resistance signal response, the

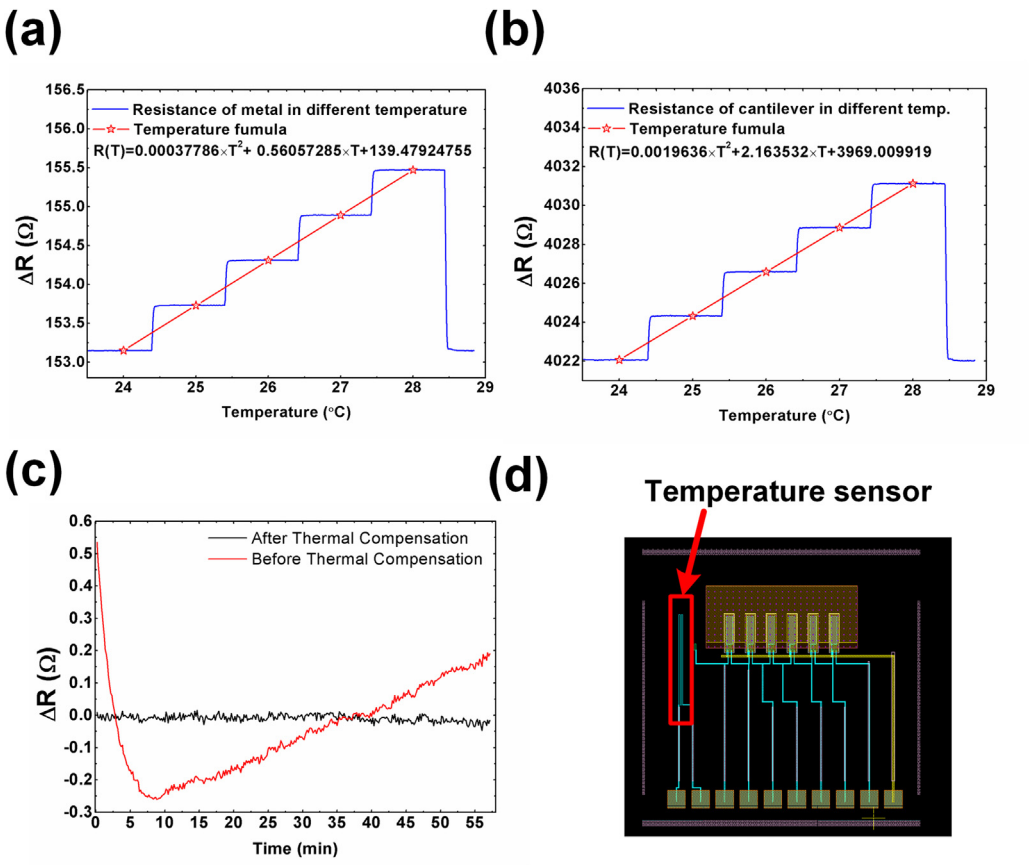

Fig. 2. (a) The sketch of aluminum thermometer resistance to temperature variation from 24 °C to 28 °C. (b) The sketch of MCL resistance to temperature variation from 24 °C to 28 °C. (c) The signal of MCL biosensor with and without thermal compensation (d) The MCL layout with the temperature sensor for MCL thermal calibration.

flat response signal, which was compared with the response signal before thermal compensation, could be measured before chemical molecule detection. In this study, the blank signal after thermal compensation was 7.85 mΩ in root mean square calculation.

2.3. Surface functionalization of the MCL sensor

The surface functionalization of the MCL sensor has several chemical and biochemical processes. Most of the chemicals and solution were purchased from Sigma-Aldrich, Inc. The antibody cannot bond on the MCL surface directly, so the self-assembly molecular (SAM) (SH $(CH_2)_7$COOH) acts as an essential bridge between the antibody and the MCL surface. At one end of the SAM of the sulfur atom, 8-Mercaptooctanoic acid bonds with the gold layer of the MCL surface by the covalent bond between aurum and sulfur (Au-S); at the other end of the SAM of the functional group is the carbonyl group which connects with the antibody by peptide bond. Firstly, we dispensed 8-Mercaptooctanoic acid with 99.7% ethanol into 100 mM. To let the SAM be stable on the gold layer of the MCL surface, the MCL was immersed in SAM solution for 24 h. Secondly, the SAM should be activated before the antibody immobilization process. To avoid leaving the non-bonded SAM on the MCL surface, the MCL should be immersed in the DI water for 10 min to remove the non-bonded SAM. The SAM was activated by a mixed solution of 400Mm of EDC (N-ethyl-N′-dimethylaminopropyl carbodiimide) and 100Mm of NHS (N-hydroxysuccinimide) in an equal ratio. As shown in Fig. 1(d), the solution was injected into the vessel for 30 min to activate the SAM biolinker. Thirdly, the gentamicin antibody (polyclonal, gentamicin, sheep IgG fraction, Abcam, Cambridge, MA, USA) was injected into the solution tank with the PBS solution concentration at 100 μg/mL in pH 8.8. Then, as a result of the chemical reaction of activating SAM, the SAM without antibody should be deactivated by enthanolamine under 1 M. Finally, after rinsing three times with ethanol, the sensor was ready for gentamicin detection.

3. Results and discussion

3.1. External electrical field for sensitivity enhancement

In this measurement, the solution was controlled to create a friendly environment for MCL surface modification and drug-antibody direct binding. To promote the response signal of drug-antibody interaction in a different drug concentration, we applied the technique of isoelectric points (pI) and electric field enhancement (Yen et al., 2009).

The isoelectric point is the pH value at which a particular molecule or surface carries no net electrical charge. For example, proteins are positively charged in a solution at a pH below the pI value and negatively charged above the pI value. In the proximity of the sensing surface, the appropriate pH solution helps both opposite charges attract the drug-antibody interaction. To select an appropriate pH environment for measurement, we investigated the pI values for both the gentamicin antibiotic and its antibody. According to a drug database, the gentamicin isoelectric point is 9.5 (HENNER and SITRIN, 1984).

A double sodium dodecyl sulfate polyacrylamide gel electrophoresis (dSDS-PAGE) was widely used to investigate the pI value of the gentamicin antibodies. By the different weight and charges on the heavy chains and light chains on the antibodies, the dSDS-PAGE can separate molecules at different speeds into a two-dimensional arrangement. In other words, at its pI value, the macromolecular capture antibody does not migrate to an electric field.

As shown in Fig. 3(b), the light chain pI values were above 6.17, and the heavy chain pI values were distributed between 5.38 and 7.79 in its pI values. As a result of the dSDS-PAGE, the range of gentamicin antibody macromolecules was simply shown to be 5.38–7.79. To obtain opposite charges between the drug and antibody, the pH environment of the PBS solution was set at pH 8.8. Both the antibody and drug molecules held opposite charges for attraction to enhance the binding capability of the MCL biosensors. Meanwhile, the antibody and the gentamicin drug respectively carried negative and positive charges in solution.

The electric field enhancement is another method to promote the response signal that is based on the technique of isoelectric points to enhance the antibody immobilization upon the MCL surface by an external electric field. The antibody immobilization condition on the gold layer of the MCL surface affects the antigen detection signal directly. Thus, to improve the rate of antibody immobilization on the gold layer for small-molecule (< 2 kDa) drug detection, the MCL biosensor utilizes the external electric field manipulation. Three steps were required. Firstly, the gentamicin pI value was found by doing 2D Electrophoresis, as shown in Fig. 3(b). Secondly, we modified the pH value of the buffer solution for the antibody. Thirdly, we defined the external electric field direction. After the condition was prepared, the different electric field strengths could be tested. We dispensed the phosphate-buffered saline (PBS) solution to pH 8.8; therefore, the carboxyl group (-COOH) on its heavy chain will generate the hydrogen ion dissociation, which makes the gentamicin antibody characterized by electron negativity. The electric field is controlled by indium tin oxide (ITO) conductive glass and metal1 which is deposited in the MCL. We define the ITO conductive glass to be negative charge and the metal1 to be positive charge for the condition in solution, as shown in Fig. 3(a). Finally, as shown in Fig. 3(c), the experiment for electric field enhancement selects three different external applied voltages, 0 V, 30 V and 60 V, respectively, with detection of the different gentamicin concentrations, to verify the feasibility of electric field enhancement to the MCL detection. Regarding the concentration of gentamicin in 20 μg/mL, the detection signals are 7.793 mΩ in 0 V, 17.107 mΩ in 30 V and 20.912 mΩ in 60 V; for the concentration of gentamicin in 35 μg/mL, the detection signals are 28.450 mΩ in 0 V, 33.621 mΩ in 30 V and 40.854 mΩ in 60 V; for the concentration of gentamicin in 50 μg/mL, the detection signals are 47.117 mΩ in 0 V, 52.130 mΩ in 30 V and 62.087 mΩ in 60 V. In summary, the average enhancing magnitude of the signal with applying an external electric field compared with that without applying electric field is 1.5 fold in 30 V, 1.8 fold in 60 V. Therefore, the antibody immobilization in 60 V electric field enhancement has optimal responses via the increasing of antibody immobilization rate on the MCL surface modification; therefore, the following gentamicin detections are based on the 60 V electric field enhancement.

3.2. Detection of gentamicin

Gentamicin ($C_{21}H_{43}N_5O_7$) drug is an aminoglycoside antibiotic with a molecular weight of 477.596 Da. Electrical detection based on nanomechanics depends solely upon the direct binding of drug-antibody recognition and biomolecular interaction, resulting in induced surface stress variation and MCL deflection. To transform the chemical reaction from antigen-antibody interaction via the MCL, the MCL underwent several surface modification processes, including SAM coating, antibody immobilization and SAM blocking to ensure the specific binding on the MCL surface. The several chemical binding processes on the MCL surface may yield the respective response signals.

As shown in Fig. 4(a), the signal response of the MCL can be signified by deflection, resistance variation, or be converted into surface stress variation. Firstly, the MCL response signal was generated by the 100 (Berger et al.,) SAM biolinker modification, resulting in signal variation to resistance and surface stress of 0.18 Ω and 0.249 N/m in compressive surface stress which displayed a bend-down deflection. As we know, there are two types of interactions of the surface stress from SAM adsorption on the MCL: surface charge redistribution and chain-chain interaction (Godin et al., 2010; Shih et al., 2013). The surface charge redistribution leads to compressive surface stress, while the chain-chain interaction generates tensile surface stress. However, the magnitude of the stress generated by surface charge redistribution is greater than the chain-chain interactions (Shih et al., 2013). According

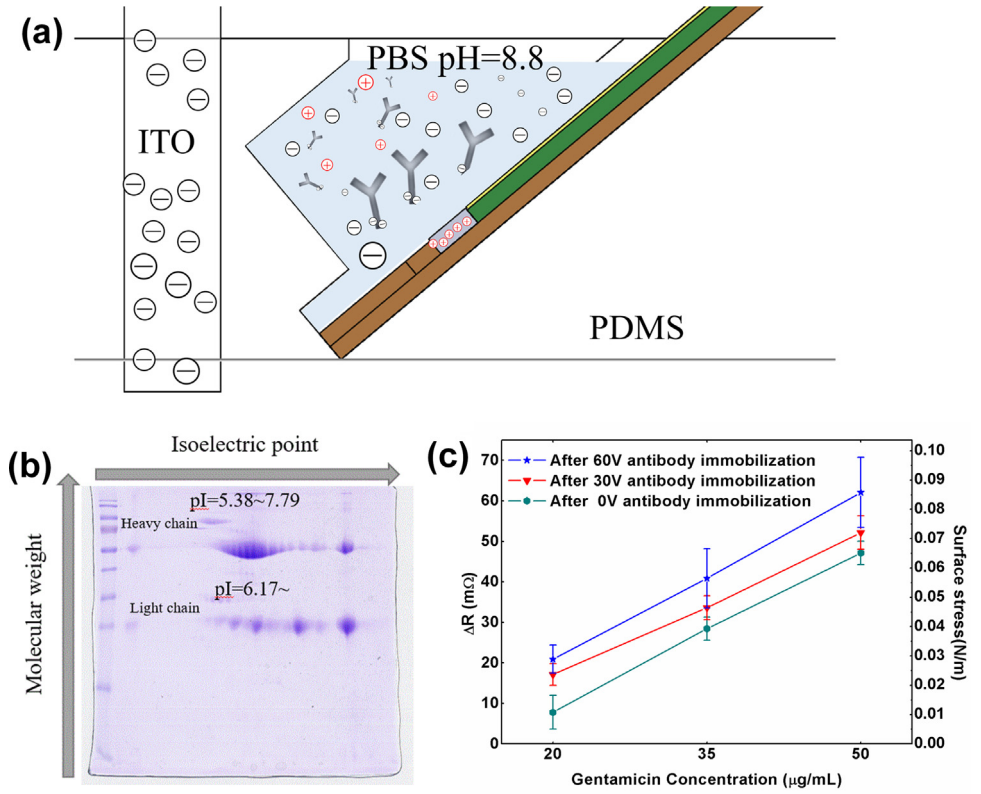

Fig. 3. (a) The schematic of the MCL biosensor under electric field enhancement. (b) The gentamicin pI value detection via dSDS-PAGE. (c) The gentamicin detection signals after three different electric fields for enhancing antibody immobilization.

to the result, the SAM adsorption on the MCL gold surface generates a compressive surface stress. Secondly, the MCL response signal was generated by the immobilization of 100 (μg/mL) anti-gentamicin molecules. The antibody immobilization was bonded randomly onto the SAM layer, resulting in signal variation to resistance and a surface stress of 0.12 Ω and 0.166 N/m in tensile stress which displayed a bend-up deflection. This indicated that immobilized antibody conformation and a molecule-level force rearrangement over a period of about 20 min achieved the required balance force on the MCL even though the immobilization process was almost 3 h. Then, we deactivated the functional SAM surface after immobilizing the antibody molecules on the MCL surface to prepare for the interaction with the target drug via direct antigen-antibody interaction.

In the last stage of target detection, the MCL response signal was generated by the specificity and interaction of the macromolecular capture antibodies with respect to 50 μg/mL gentamicin drug in PBS solution with pH 8.8. It took approximately 15 min for the steady-state signal to achieve a molecular force equilibrium state. This direct binding generated a considerable deflection of induced surface stresses which could result from a gradual rearrangement of the antibody conformation. In this study, the gentamicin drug was first investigated using the MCL biosensor technique.

3.3. Sensitivity of the MCL gentamicin sensor

The result in Fig. 4(b) shows the response signal via resistance variation in different gentamicin concentrations with 60 V external electric field enhancement. According to different gentamicin concentrations, 10 μg/mL 20 μg/mL, 35 μg/mL, 50 μg/mL, 150 μg/mL and 200 μg/mL, respectively, the response signals displayed 5.030 mΩ, 20.912 mΩ, 40.854 mΩ, 65.694 mΩ, 103.228 mΩ and 112.194 mΩ, respectively. To confirm from antigen-antibody specific binding, there

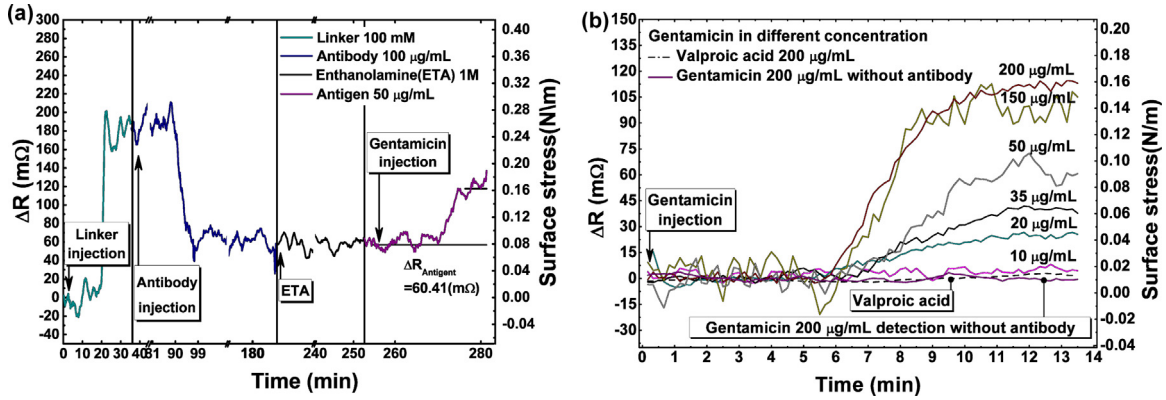

Fig. 4. (a) An overall detected signal of the SAM biolinker, immobilized capture antibody, SAM deactivation and gentamicin detection. (b) The gentamicin detection in different concentrations, the specificity test by valproic acid as well as control experiment by gentamicin detection without capture antibody.

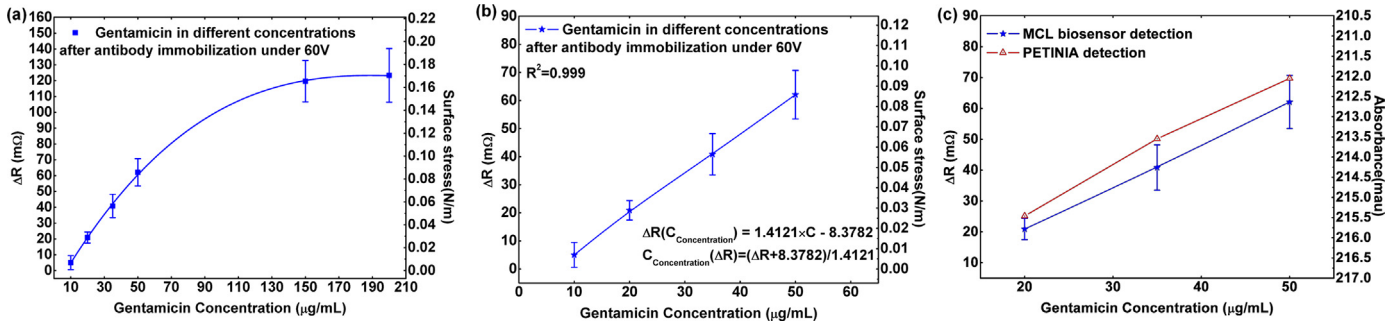

Fig. 5. (a) The fitting curve of the gentamicin detection in different concentrations via 60 V antibody immobilization. (b) The linear region of gentamicin detection with perfect fit with the measurement data via $R^2 = 0.99$, under 60 V antibody immobilization with the relation between the gentamicin concentration and the MCL resistance variation or surface stress difference. (c) The comparison between MCL biosensor and PETINIA, the most widely used instrument in hospital, shows the high correlation in gentamicin drug detection.

was an additional valproic acid drug detection with a 200 μg/mL high concentration. The measurement showed that there was no response between the gentamicin-antibody and the valproic acid drug. Another control test was performed with the SAM deactivated before antibody immobilization (supporting information Figure S1), the measurement result showed also no response (Fig. 4(b)). Therefore, following the result shown in Fig. 4(b), we can sketch the fitting curve for different gentamicin concentration response signals between 10 and 200 μg/mL corresponding to the resistance variation or the surface stress difference, as shown in Fig. 5(a). Hence, here we found the linear region between 10 and 50 μg/mL gentamicin concentration with linear regression which showed a perfect fit with the measurement data via $R^2 = 0.99$, as shown in Fig. 5(b). According to the fitting calibration curve, we established an equation of $\Delta R = 1.4121 \times C_{Concentration} - 8.3782$ between the gentamicin concentration $C_{Concentration}$ and the MCL response signals ΔR to be a mathematical relationship for concentration prediction after the MCL response signal in gentamicin detection.

In this work, the magnitude of the mean of the blank signal with the thermal compensation has been measured is 3.33 mΩ after signals processing root-mean-square (RMS). The magnitude of the standard deviation obtained from blank signals of MCL biosensor is 0.54 mΩ (detail explanation and calculation was described in Supporting information). Limit of detection (LOD) was expressed as the mean of the blank signal plus three times standard deviation. Therefore, the LOD of the MCL biosensor for gentamicin detection is 4.95 mΩ and expressed as the concentration of 9.44 μg/mL by the calibration curve. As the result, the MCL in this study has great potential to be a point of care and therapeutic drug measurement biomedical device via its minimum concentration detection of 9.44 μg/mL and its minimum surface stress detection of 0.0068 N/m in the gentamicin drug.

3.4. Comparison with existing method

PETINIA, a quantitative analysis technology in immunology, is the most commercially used with immune-system disease (Opheim et al., 1984). The detection principle of PETINIA immerses latex molecules and antigens in the same cavity with the antibody to undergo competitive binding. The turbidity in the cavity may rise when most of the antibody binds with latex molecules with a low antigen concentration. On the contrary, the turbidity in the cavity may decrease when most of the antibody binds with the antigen with a high antigen concentration. To analyze the difference between different turbidity caused by a different antigen concentration, the optical method of transmission turbidimetry has been applied. Therefore, the peritonitis therapeutic drug gentamicin is widely detected by PETINIA in hospital to monitor the drug concentration in the patient's blood. However, PETINIA has several drawbacks with respect to point of care: it is a huge instrument, it is expensive, it requires a large sample, it requires skill and it is time-consuming. In contrast, the MCL sensor has several advantages over PETINIA with respect to point of care, and the MCL sensor only needs 15 min at most to complete the detection. As shown in Fig. 5(c), the gentamicin concentration detection within three different concentrations in the MCL sensor and PETINIA had a high correlation, showing that the MCL has great potential to take the place of PETINIA in gentamicin detection.

Moreover, there are several detection methods of gentamicin in the Table 1 below. It was found that common advantages of optical type of methods are high sensitivity, high accuracy and broad specificity ability to detect the complex mixtures. The merit of the electrochemical type biosensors are rapid detection and simple preparation process. However, these methods are all not suitable for label-free and portable usage. It is an undeniable fact that the sensitivity of MCL sensor was not high compared to other methods, but the linear range and LOD are enough for meaningful clinic measurement. Therefore, in this work, MCL sensor demonstrated the great potential to be a low-cost, portable, label-free and real-time monitoring device for POCT instrument development.

4. Conclusion

We successfully achieved quantitative analysis of gentamicin reaction with various concentrations by applying the CMOS-BioMEMS piezoresistive MCL-based sensor. The sensor displayed appropriate responses in the therapeutic range of drug concentration in the liquid samples. With the electric field enhancement, the piezoresistive MCL sensor increased the detection sensitivity to detect more low-concentration gentamicin drug. We developed a directly titration method to replace the microfluidic system and hold the advantage of the microscale specimen (10 μL) to solve the problem of slow velocity of fluid in the microchannel. Although detection limit of the MCL biosensor was 9.44 μg/mL and linear range of detection concentration was only 10–50 μg/mL, the sensor was considered to have capability for therapeutic monitoring of the patient who takes the gentamicin drug. The sensitivity of the sensor can be improved by a well design of length and shape of piezoresistor of MCL. This work was also compared with the most widely used instrument in hospital and showed a strong correlation in gentamicin detection. The MCL sensor showed high feasibility for detecting gentamicin in clinical application, but had an urgent need of performing itself in real human serum and blood samples.

Acknowledgements

The authors acknowledge the financial support of the Ministry of Science and Technology, Taiwan (MOST 105-2221-E-027-140-MY2) and the manufacturing help from the Nano-Electro-Mechanical-Systems (NEMS) Research Center, National Taiwan University. The authors acknowledge the experimental advice and guidance from Professor Long-Sun Huang at National Taiwan University. The authors acknowledge

Table 1
An overview on recently reported methods for determination of gentamicin.

Method	Type	Sample	Linear range (μg/mL)	LOD (μg/mL)	Advantage	Disadvantage	Reference
[a]MCL-based sensor	Mechanical	PBS	10–50 (μg/mL)	9.44 (μg/mL)	Label free, portable, rapid response, real time detection	High noise level	This work
[b]PETINIA	Optical	Serum	0–46 (μg/mL)	–	Sensitive and sufficient accuracy	Time-consuming Costly equipment	(Opheim et al., 1984)
[c]ELISA	Optical	Serum	0.002–0.5 (μg/mL)	–	Protein-rich sample, high sensitivity, broad specificity	Expensive, not real-time, Label	(Odekerken et al., 2015)
[d]SPE/ PR-HPLC	Optical		0.5–10 (μg/mL)	75×10^{-3} (μg/mL)	Sensitive and sufficient accuracy	Time-consuming Costly equipment Unportable	(Al-Amoud et al., 2002)
[e]SPE-CE	Optical	Serum	0.25–6.9 (μg/mL)	0.12 (μg/mL)	Simple, high selective, accurate, repeatable and UV detector	Time-consuming Costly equipment Unportable	(Kaale et al., 2005)
[f]Fluorimetry (NBD-Cl)	Optical	plasma	0.84–4.2 (μg/mL)	0.112 (μg/mL)	simple, rapid and sensitive fluorimetric	Label	(Al-Majed et al., 2003)
Potentiometric	Electrochemistry	plasma	$1.19–4.77 \times 10^{3}$ (μg/mL)	0.477 (μg/mL)	Short response time, simplicity, cheap and quick preparation process	Limited anodic range, pH range limitation	(Khalil and Abed El-aziz, 2016)

[a] MCL, microcantilever.
[b] PETINIA, particle-enhanced turbidimetric inhibition immunoassay.
[c] ELISA, enzyme-linked immunosorbent assay.
[d] SPE/ PR-HPLC, solid-phase extraction/ reversed phase high-performance liquid chromatography.
[e] SPE-CE, Solid-phase extraction-capillary electrophoresis.
[f] NBD-Cl, 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Mr. Wei-Je Chen's assistance with the getamicin measurement using the commercial instrument.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2018.09.014.

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