Biosensors and Bioelectronics 59 (2014) 233–238

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Detection of the antiepileptic drug phenytoin using a single free-standing piezoresistive microcantilever for therapeutic drug monitoring

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ARTICLE INFO

Article history:
Received 25 November 2013
Received in revised form
17 March 2014
Accepted 24 March 2014
Available online 28 March 2014

Keywords:
Antiepileptic drug
Phenytoin
Piezoresistive microcantilever
Serum
Therapeutic drug monitoring

ABSTRACT

Phenytoin, one of the most widely used antiepileptic drugs, suppresses the abnormal brain activity often seen in seizures. In this study, we report the electrical detection of phenytoin as an antiepileptic medication with a narrow therapeutic dosage range to which therapeutic drug monitoring (TDM) is applied. The measurement technique used an electrical detection of a piezoresistive microcantilever biosensor. This label-free, electrically measured microcantilever can be miniaturized in order to be portable for point-of-care, personal diagnosis or for personalized therapeutic drug monitoring. The miniaturized piezoresistive microcantilever was fabricated by micro-electro-mechanical system processes, and was integrated into a microfluidic channel with a system for label-free detection. The microcantilever biosensor was approved for the detection of phenytoin in solutions of deionized water and 100% fetal bovine serum. A linear profile in a drug-concentration range of 10–80 μg/mL was detected, with the signal resolution being about 0.005 Ω. The concentration sensitivity was 2.94×10^{-6} (μg/mL)$^{-1}$. The binding affinity (K_D) was calculated to be 58 μg/mL. The results of the present piezoresistive microcantilever biosensors showed a solid correlation of phenytoin drug detection with that in the clinically used fluorescence polarization immunoassay (FPIA).

1. Introduction

Adverse drug reaction has recently received increasing attention. For some specific drugs with narrow therapeutic ranges, the use of therapeutic drug monitoring (TDM) may reduce adverse drug reactions so that drug toxicity can be avoided (Jaquenoud Sirot et al., 2006). TDM is a clinical method to measure specific drug concentrations in the blood, and to determine drug dose and usage. Antiepileptic medications have been commonly managed by TDM to treat and prevent seizures. The introduction of TDM to patients makes it possible to optimize efficacy and avoid toxicity (Neels et al., 2004).

Phenytoin, one of the antiepileptic drugs, has a narrow reference range of 10–20 μg/mL, which is most frequently used with TDM (Warner et al., 1998). Moreover, in previous studies of adverse drug reactions associated with antiepileptic drugs, phenytoin was used in the highest percentage of the reported cases of these adverse drug reactions (Roopa et al., 2008).

Drug analysis in blood or serum is commonly performed in a clinical laboratory using the fluorescence polarization immunoassay (FPIA) (Külpmann et al., 1984). This measurement technique requires skilled operating technicians, a milliliter-scale sample and reagent volumes, and long turnaround times. Patient's individual therapeutic concentration was proposed (Perucca, 2000), because a wide variability among individuals' serum concentrations was observed. However, there are no simple diagnostic or laboratory tests for seizure disorders.

Microcantilever-based sensors have received great attention in fields such as biological studies (Lavrik et al., 2004), clinical diagnosis (Guanghua et al., 2001; Wee et al., 2005), environmental monitoring (Datskos and Sauers, 1999; Berger et al., 1997), functional genomics (Fritz et al., 2000; Mukhopadhyay et al., 2005), and pharmacological drug screening (Zhang et al., 2004). Light in weight, free of fluorescence labeling, and highly compatible with integrated circuits, the microcantilever label-free biosensing technique provides real-time investigation, high sensitivity, and miniaturization. Hence, it is potentially portable for personal diagnosis or for use as a point-of-care platform. Vancomycin, one of the

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http://dx.doi.org/10.1016/j.bios.2014.03.047
0956-5663/

antibiotics with which TDM is used, was first measured using the microcantilever technique (Ndieyira et al., 2008).

The common method to detect the cantilever deflection can be employed using an optical-level technique (Ndieyira et al., 2008) or electrical detection (Wee et al., 2005; Yen et al., 2013; Yoshikawa et al., 2011; Shekhawat et al., 2006). In contrast to an optical-level instrumentation that requires space and alignment for laser, optics, and detectors, the electrical transducer converting a microcantilever deflection into an electrical signal can be miniaturized to make it portable for point-of-care or personal diagnosis.

In biosensing applications, the dual-beam, free-standing microcantilever configuration has been extensively used for detection of chemical interaction and biomolecular recognition in a liquid environment. This work employs a single free-standing, thermally controlled piezoresistive microcantilever to circumvent the unexpected chemical disturbances for biochemical measurement (Yen et al., 2013).

In this study, phenytoin is measured using the single free-standing, thermally controlled piezoresistive microcantilever. Single free-standing piezoresistive microcantilevers and microchannels are micromachined, packaged, and assembled in a small-form fashion. Measurement of the microfluidic microcantilever biosensor is conducted in a thermally controlled environment within 0.1 °C. Various concentrations of phenytoin are measured both in deionized water and in serum of a complex liquid environment. Measurement of phenytoin is also performed using the conventional technique of FPIA in comparison with the microcantilever biosensors.

2. Materials and methods

2.1. Use of a single free-standing piezoresistive microcantilever

The electrical piezoresistance-based transducer of a microcantilever deflection is miniaturized to make it portable for point-of-care or personal diagnosis. The piezoresistive microcantilever that incorporates biomolecular receptors to bind with specific molecules is employed to yield a deflection and thus an associated resistance change due to the molecular recognition. In a commonly used sensing arrangement of conventional dual cantilevers in the Wheatstone bridge circuit, both reference and additionally gold-coated sensing cantilevers are shown to inherently have heterogeneous surface material and different multilayer structures. Prior to immobilization of any capture protein molecules, these two different free-standing cantilevers may yield small, independent deflections in response to their flowing field, temperature fluctuation, or chemical substances. Moreover, the capture-antibody-immobilized sensing microcantilever is highly sensitive to a pH change in solution. A single unknown or multiple-chemical solution in a dual-beam arrangement may lead to unexpected or irreproducible results. To resolve the problem of independent responses generated by dual cantilevers in chemical interaction or biomolecular recognition, the single free-standing cantilever arrangement for detection is employed in this study to avoid physical or chemical interferences (Yen et al., 2013). However, the trade-off in the single-beam sensing arrangement arises in a cantilever temperature-sensitive effect. Therefore, a constant-temperature, heat-insulted enclosed system that contains the sensor device is used in this study.

2.2. Design and fabrication of the piezoresistive microcantilever

The piezoresistive microcantilever is composed of several layers, including a structural insulation layer of nitride (Si_xN_y) and oxide, a piezoresistive polysilicon layer, an insulation layer of nitride, and a gold layer for surface reaction. The released microcantilever must be flat and straight. Therefore, the built-in stress between the layers of a microcantilever is considered to balance the residual stresses of the multiple layers. The initial curvature of the released microcantilever may yield the flow-induced disturbance of a noise signal. As previously studied in the context of a low vibration deflection noise, the microcantilever shape is kept as thin and straight as possible.

In addition, placement of a piezoresistive layer from the neutral axis of the beam is related to sensor sensitivity upon stress occurrence across the beam. The neutral axis of a beam is an axis in the cross section along which there are no longitudinal stresses or strains. A great distance created by the thickness between the piezoresistive layer and the beam's neutral axis is desired to yield high stress or strain. The dimensions of the microcantilevers are designed in this study to be 200 μm long, 60 μm wide and about 1.2 μm thick. To gain the maximum stress in deflection, the neutral axis Z_N in this study is calculated to be 585.65 nm; this is located on the structural silicon nitride layer.

Fig. 1(a) shows the microcantilever cross-sectional view and its associated processes. A 500 μm-thick p-type ⟨1 0 0⟩ silicon wafer was used as a starting substrate. First, a 215-nm low-pressure chemical vapor deposition (LPCVD) low-stress nitride layer was deposited on both the top and bottom sides of a Si wafer at 780 °C. Meanwhile, the top layer was used as a protective piezoresistive polysilicon layer in a later KOH backside wet etching, and the bottom layer acted as a blocking mask for KOH wet etching. Then, a 400-nm SiO_2 film was deposited on the Si_3N_4 top layer using a plasma-enhanced chemical vapor deposition (PECVD) technique to be made in an overall residual stress balance after release. The overall residual stress balance allowed a released microcantilever to be as flat as possible for minimum curvature to significantly reduce the flow-induced disturbance. A 120-nm polysilicon layer was deposited by LPCVD, followed by a boron-doped ion implantation with 20 keV in energy, 5.437×10^{19} cm^{-3} in doping concentration, and by annealing at 1080 °C. A reactive-ion etching (RIE) was used to define the patterned polysilicon layer for a piezoresistive effect. Subsequently, 15-nm chromium (Cr) and 150-nm gold (Au) were evaporated on the polysilicon layer to be electrically connected for wire bonding. Meanwhile, a Cr layer was

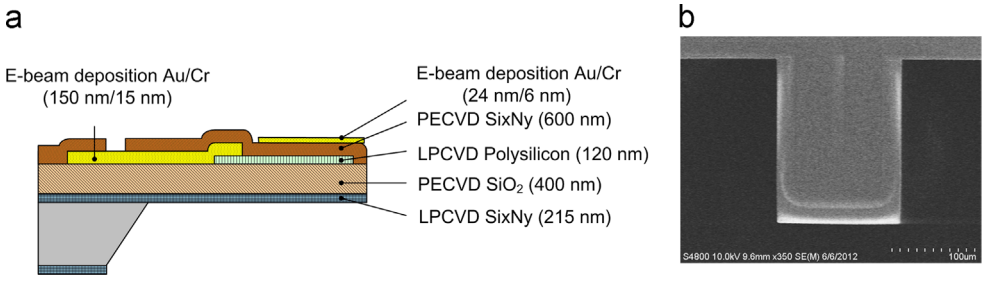

Fig. 1. (a) The piezoresistive microcantilever biosensor device and its associated process. (b) A scanning electron microscope image of a microcantilever.

used to act as an interfacial layer to re-form an adhesive force for a gold layer. A 600-nm plasma-enhanced chemical vapor deposition (PECVD) nitride film was used as a passivation layer to protect a structure being etched by a following backside anisotropic wet etching, and to prevent electrical interference from an ionic solution entering the electrically conductive piezoresistive layer.

To create a biosensing surface of a microcantilever, the gold material was exposed in solution because of its excellent binding property with sulfur (S), which is part of a terminal biolinker group. For biochemical surface treatment, 6-nm chromium and 24-nm gold layers were deposited. Lastly, a 30% potassium hydroxide (KOH) solution was used to release a microcantilever. Fig. 1(b) depicts an SEM image of the complete piezoresistive microcantilever.

2.3. Package and assembly

The overall microfluidic biosensing packaged device consisted of a piezoresistive microcantilever sensor chip, a microchannel with a micromachined silicon bottom pedestal and a PDMS-based microchannel capping structure, and a PCB with essential electronic components. A diced microcantilever chip was attached onto an electrical PC board (PCB) and placed inside a microchannel. The microchannel that allowed liquid solution to flow over the microcantilever was designed in a rectangular cuboid 14.8 mm long, 2.4 mm wide, and 100 μm high with a total volume of 3.55 μl. The microchannel consisted of a micromachined silicon microchannel substrate and a poly-dimethylsiloxane (PDMS) capping channel structure. Meanwhile, a poly-dimethylsiloxane (PDMS) was used to make a microchannel capping structure by replication from a micromachined silicon mold. The last step was to assemble all parts together onto a circuit board.

The piezoresistive temperature sensor was embedded into the sensor device to provide real-time temperature monitoring during the measurement. The entire package provided the sensor with a small stable liquid chamber for biological and chemical reactions. The assembled package was placed in a temperature-controlled, heat-insulated cryostat system. The cryostat system consisted of a heat-insulating box and a cryostat tank. The volume of the system was 12 cm long, 8 cm wide and 5 cm high. The system was designed to be heat insulated with the outside environment, and to be temperature-controlled within $\pm 0.1\ ^{\circ}C$.

2.4. Surface modification

After the packaged device was made, a thorough cleaning was required for the sensing surface to be reactive in surface treatment. The surface-cleaning process started with a rinsing with deionized water (DI water), followed by 1 M hydrochloric acid (HCL), DI water, 1 M sodium hydroxide (NaOH), DI water, 75% ethanol and DI water.

To serve as a microcantilever biosensor, the physical sensing surface was required to experience several major steps involving chemical and biochemical processes on its solid surface. Meanwhile, the selected concentration of capture antibodies for immobilization was taken into account in this study, possibly affecting the electrostatic adsorption in the immobilization process.

First, a self-assembly monolayer (SAM) solution of 100 mM of 8-Mercaptooctanoic acid ($SH(CH_2)_7COOH$) was injected into the microchannel of the microcantilever biosensor and was incubated for 12 h to achieve a dense alignment. The molecular weight of 8-Mercaptooctanoic acid is approximately 176.28. The sulfur atom of one end in SAM was able to covalently react with the gold surface, and the other functional terminal of the carboxylic group in SAM provided antibody attachment with a peptide bond. The solution was made in a mixture with 75% ethanol solvent. After the linker molecules were covalently chemisorbed on the gold-coated surface of the microcantilever, this reactive self-assembled monolayer was activated using a mixed solution of EDC and NHS. The solution of 75 mg/mL of EDC (N-ethyl-N′-dimethylaminopropyl carbodiimide) and 11.5 mg/mL of NHS (N-hydroxysuccinimide) in an equal ratio was injected into the microchannel for 40 min to activate the SAM biolinker. A continuous-flow pump was filled with distilled phosphate-buffered saline (PBS) at pH 7.2. After the activation of EDC/NHS to enhance a binding of antibody molecules and SAM biolinker, 300 μL of the capture protein (monoclonal anti-phenytoin, mouse IgG1 kappa, Abcam ab21785) was then injected, followed by 100 μL of ethanolamine hydrochloride solution. Finally, the small molecule analyte of phenytoin, used as the antiepileptic drug, was injected for detection. The NI™ multichannel acquisition system and the LabVIEW™ software were used to read out the resistances of piezoresistive microcantilevers for display.

3. Results and discussion

3.1. Detection of phenytoin

Phenytoin, which has a complex metabolism, has been known to induce the expression of hepatic drug-metabolizing enzymes. Phenytoin empirical formula is $C_{15}H_{12}N_2O_2$, and its molecular weight is 252.3 Da. In the serum or plasma of a complex environment, the protein binding of the drug is featured to reduce the presence of the free phenytoin drug.

To serve as a biosensor, the device was required to experience several major steps involving chemical and biochemical processes. As used in line with commercial surface plasmon resonance biosensors, an appropriate capture antibody concentration for the immobilization is recommended to be in a range between 10 μg/mL and 200 μg/mL. In this study, the immobilized capture antibody concentration of 100 μg/mL was chosen.

Fig. 2(a) depicts an overall plot of the microcantilever deflection in response to three major steps of the chemical and biochemical processes. The response of the microcantilever can be obtained in deflections and its associated resistance changes, or gets converted into induced surface stresses. Meanwhile, the first stage was the response signal of the 100 mM SAM biolinker. The changes in resistance and surface stress were 0.12 Ω and 0.85 N/m in compressive stress. As demonstrated in Fig. 2(a), the second stage was the response signal of the immobilization of 100 μg/mL anti-phenytoin molecules. The capture antibody molecules were bonded randomly onto the SAM layer. The induced change in surface stress was measured as 0.35 Ω and 1.6 N/m in tensile stress, which displayed a bend-down deflection. This indicated that capture antibody conformation and a molecule-level force rearrangement over about 50 min achieved the required balance force on the microcantilever. After inactivation or blocking of the functional SAM surface, the immobilized antibody molecules on the microcantilever surface were then ready for interaction with the target drug. Therefore, the microcantilever was modified to be capable of sensing specific small molecules of the target drug.

In the last stage, the antiepileptic drug phenytoin in de-ionized water solution was the target for measurement. Small molecular phenytoin has a minute molecular weight of 252.3 Da in comparison with around 150 kDa for macromolecular capture antibody. In this study, the antiepileptic phenytoin drug was first investigated using the microcantilever biosensor technique. The measurement began with the deionized water solution to circumvent the interference of impurities and unknown ions. The signal of the last stage was generated by the specificity and interaction of the macromolecular capture antibodies with respect to the small

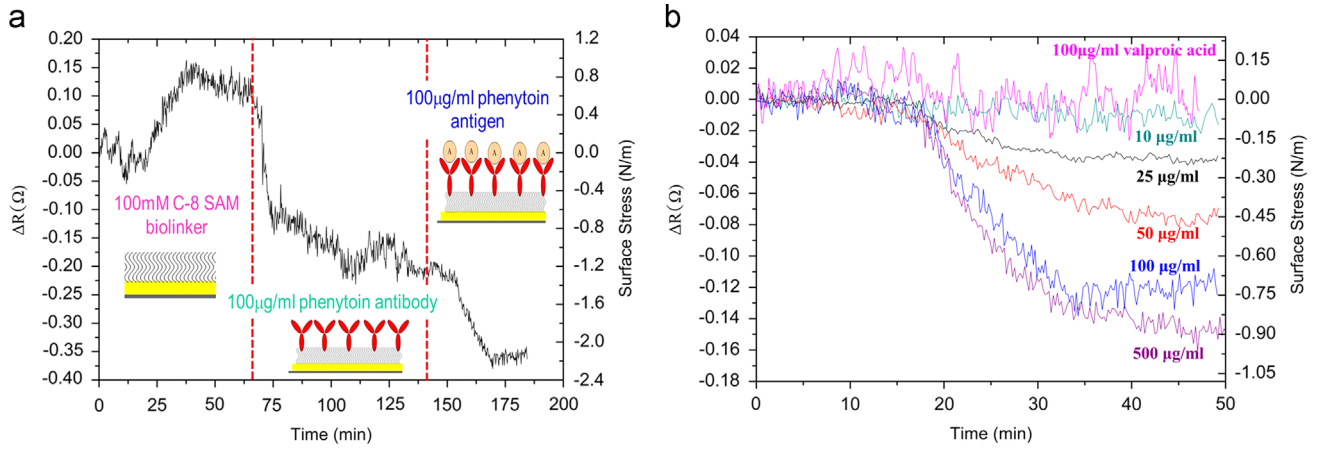

Fig. 2. (a) An overall detected signal of the SAM biolinker, immobilized capture antibody, and phenytoin drug. (b) Detection of various concentrations of phenytoin drug between 10 and 500 μg/mL in DI water.

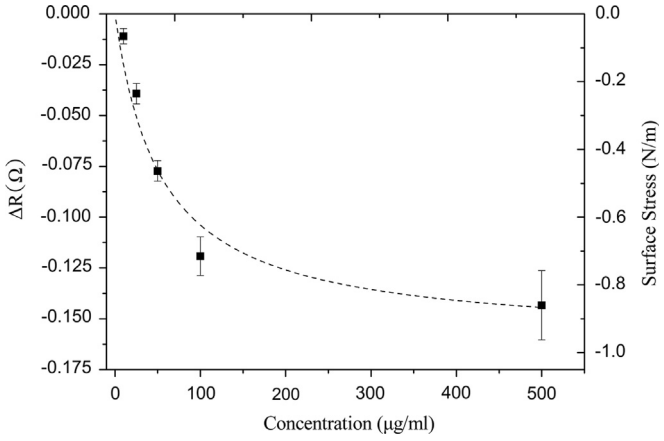

Fig. 3. The relationship between the change in resistance and the various concentrations of the phenytoin drug.

molecular target drug of 100 μg/mL phenytoin in deionized water solution. It took approximately 20 min to achieve a molecular force equilibrium state of the steady-state signal. The continual deflection change prior to the equilibrium state could result from a gradual rearrangement of the antibody conformation.

As illustrated in Fig. 2(b), the result showed the electrical responses in resistance change of the microcantilever biosensors with respect to various phenytoin concentrations of the target drug between 10 μg/mL and 500 μg/mL, which covered the therapeutic range of 10–20 μg/mL. As the phenytoin continued to increase the concentration in solution, the steady-state response signals also increased in resistance change. The signal resolution was around 0.005 Ω in resistance change. In order to verify specific molecular recognition of the capture-antibody-coated microcantilever biosensors, we employed 100 μg/mL of another important antiepileptic drug, valproic acid; it also requires careful TDM. Unlike phenytoin in antiepileptic medications that enhance the expression of the liver, valproic acid, which inhibits multiple liver enzymes, has been known to cause drug–drug interactions with other antiepileptic medications (Neels et al., 2004). The response signal of the microcantilever biosensor was found to be negligible in the presence of valproic acid in DI water solution. This ensured the high specificity of the microcantilever biosensors for drug detection in antiepileptic therapeutic drug monitoring.

To investigate the reproducibility of microcantilever biosensors for drug detection, we performed a series of measurement experiments. As shown in Fig. 3, an average of steady-state resistance changes at each concentration was obtained in three tests. The result showed the resistance change increase with an increase in the phenytoin concentration. As shown in Fig. 3, the dynamic range and sensitivity were also investigated by varying the phenytoin concentrations in DI water. The plot showed an approximately linear variation in a range between 10 and ~80 μg/mL. Meanwhile, the linear regression equation was $\Delta R\ (\Omega) = -0.002[C]\ (\mu g/mL) + 0.004$ with a linear correlation coefficient of 0.997. Based on the system noise (0.005 Ω) described previously, the estimated limit of detection (LOD) defined as the analyte concentration corresponding to a signal-to-noise ratio of 3 was calculated to be 9.5 μg/mL. Moreover, the sensor response was obtained in $(\Delta R/R)/[C]\ (\mu g/mL)$ to be $2.94 \times 10^{-6}\ (\mu g/mL)^{-1}$. The profile reached a plateau beyond the concentration of 100 μg/mL. As reported previously, the therapeutic range of phenytoin was suggested to be 10–20 μg/mL. This linear profile lay within this clinically relevant therapeutic range of phenytoin, which was important for pharmacokinetic or dynamic drug profiling and personalized medicine.

This plot may illustrate the quantitative biomolecular binding that accounts for drug–target binding events. In a previously studied report, the binding affinity of antibiotic vancomycin, which has long been a vital therapeutic drug for the treatment of infections caused by the Gram-positive bacteria, was obtained using this label-free microcantilever technique (Ndieyira et al., 2008). The kinetics of biomolecular binding on microcantilever surfaces can be described using a simple Langmuir first-order scheme. The model assumes that the molecules are adsorbed at a fixed number of well-defined sites, each of which is energetically equivalent (a random process) and occupied by complete monolayer coverage when the surface reaches the saturation level. Meanwhile, the rate constant for binding kinetics (association) is given by k_a, whereas the rate constant for unbinding kinetics (dissociation) is given by k_d. The rate of binding between the capture antibodies and small molecules was given due to the random conjugation by the following equation (Kuan et al., 2012):

$$\frac{dN}{dt} = k_a \Phi(N) C_s - k_d N \tag{4}$$

N is the number of binding on the microcantilever surface as a function of time, C_s is the phenytoin drug concentration on the sensing surface, and $\Phi(N)$ gives the remaining pairs available for binding. Meanwhile, the capture protein in this study was used with mouse monoclonal anti-human phenytoin IgG1 molecules (Abcam ab21785). For a small ratio of k_d/k_a with high affinity antibody–drug interaction, the second term on the right-hand side

indicating the dissociation process can be disregarded. We assumed that the response of the microcantilever from the antibody–drug interaction could be effectively attributed to the generation of surface stress. In practice, the correlation could be described in an equilibrium state by an input of $dN/dt=0$, and in terms of the saturation level in the equilibrium state to derive another form of kinetic-based systems.

$$\sigma_{eq.} = \frac{\sigma_{max} C_s}{K_D + C_s} \quad (5)$$

Since σ_{eq} and σ_{max} represent the equilibrium (steady state) and maximum values of surface stress, respectively; K_D is equal to k_d/k_a and stands for the equilibrium dissociation constant or binding affinity. As σ_{eq} is set to be at $\sigma_{max}/2$, K_D can be determined to be equal to C_s. In this study, the binding affinity (K_D) was calculated to be 58 μg/mL.

3.2. Detection of phenytoin in serum

As mentioned earlier, the protein binding of drug in a protein-abundant serum or plasma of a complex environment is featured to reduce the presence of the free phenytoin drug. In a conventional detection of phenytoin or other drugs, the fluorescence polarization immunoassay (FPIA) widely used nowadays is conducted in fetal bovine serum (FBS). Serum is blood plasma from which the clotting factors are removed. Serum carries proteins (mostly albumin, immunoglobulins, interferon etc.), electrolytes, antibodies, antigens, hormones, and drugs (Adkins et al., 2002). In this study, the miniaturized microcantilever biosensor was tested for drug detection under a complex fetal bovine serum environment to determine whether any small molecules or macromolecules blocked or obstructed the interaction and binding between the antibody and the phenytoin drug. Two approaches were used in this study to aid the detection in fetal bovine serum. One was to increase the surface coverage of the capture antibody onto the microcantilever sensing area. Increased with the injection of the capture antibody concentration from 100 μg/mL to 300 μg/mL in fetal bovine serum, the microcantilever sensing surface provided target drug molecules with more accessible drug-binding sites. This effect was proven in our previous studies (Yen et al., 2009). Secondly, by controlling the pH of the serum environment, we may adjust both the antibody and the drug target into opposite charges owing to their own isoelectric points (pI) (Pei et al., 2010). The isoelectric point is the pH at which a particular molecule or surface carries no net electrical charge. In other words, macromolecular capture antibody at its pI value does not migrate in an electric field.

In order to select an appropriate pH environment for measurement, we investigated the pI values for both phenytoin and its antibody. According to the report in a drug database, the isoelectric point is 8.33 (Hara et al., 1999).

As for anti-phenytoin molecules, a double sodium dodecyl sulfate polyacrylamide gel electrophoresis (dSDS-PAGE) was used to calibrate its pI value. A general structure of all antibodies is quite similar. However, the terminal tip of a Y-shape antibody protein has a different binding sites that allows the antibody to attach to its specific types of antigens. The Y-shaped protein comprises four peptide chains. These are two identical heavy chains and two identical light chains connected by disulfide bonds. With different weights and charges that travel at different speeds, the dSDS-PAGE is able to separate molecules into a two-dimensional arrangement. As shown in Fig. 4(a), the light chain that denoted the molecular weight of 20 kDa had 6.0–6.9 pI values, and the heavy chain with the molecular weight of 75 kDa had 6.7–7.6 pI values. As a result, in the dSDS-PAGE, the range of phenytoin antibody macromolecules was simply shown to be 6.0–7.6. In order to have opposite charges of the phenytoin drug present, the pH environment of the serum was chosen to be pH 8.05. Both the antibody and drug molecules exhibited opposite charges for attraction to enhance the binding capability of microcantilever biosensors. Meanwhile, the antibody was positive and the phenytoin drug was negative in this serum environment.

As reflected in Fig. 4(b), these signals showed the responses in the detection of 100 μg/mL phenytoin drug in three solutions, these being pure DI water, 50% FBS with 50% DI water, and 100% FBS. As expected, the response signals in three environments exhibited similar profiles over the time period. In DI water, the microcantilever was significantly deflected. In contrast to a pure solution in DI water, the response signals were considerably reduced in the presence of a complex serum environment that carried albumin, immunoglobulins, electrolytes, antibodies, antigens, hormones, and drugs. In this study, the resistance change of 0.04 Ω in 100% serum was even lower than that of 0.06 Ω in 50% serum. The surface stresses were 0.24 N/m in 100% serum and 0.36 N/m in 50% serum, respectively. The presence of a complex environment as well as relevant protein binding of the free phenytoin drug in serum considerably reduced the interaction between the antibody and the phenytoin drug.

Fig. 5 exhibits the comparison results of phenytoin drug detection by use of the present piezoresistive microcantilever biosensor

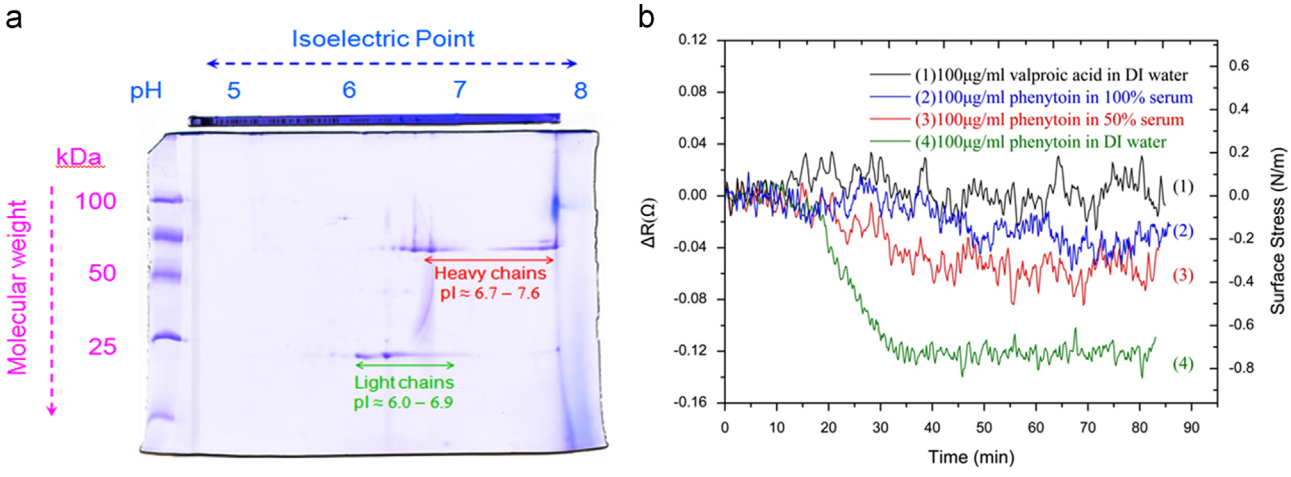

Fig. 4. (a) The 2D-SDS-PAGE result of phenytoin antibody. (b) The phenytoin drug detection using the present microcantilever biosensors was successfully demonstrated in a complex body-fluid environment of serum that contained proteins, hormones, drugs, antibodies, antigens, and other components.

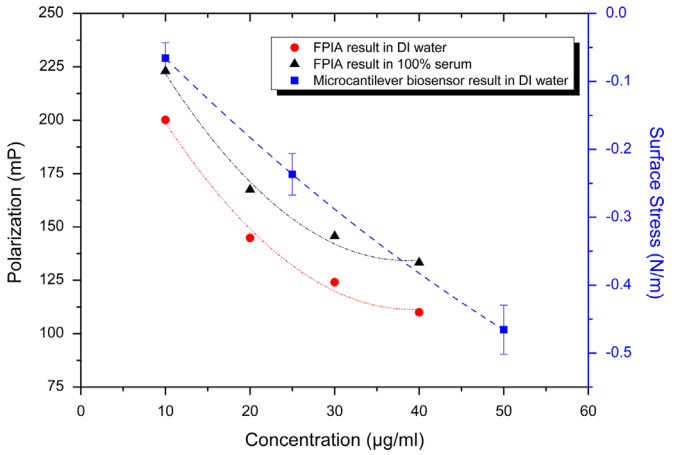

Fig. 5. The similar tendency of increasing phenytoin drug concentrations in detection utilizing the traditional FPIA technique and the present piezoresistive microcantilever.

and the clinical diagnosis using the fluorescence polarization immunoassay (FPIA). FPIAs are homogeneous, single-step assays suited for high-throughput screening of large numbers of samples. This instrument, generally used in a clinical laboratory, requires skilled operating technicians, milliliter-scale samples and reagent volumes, and long turnaround times. FPIAs are based on the competition of fluorophore-labeled phenytoin with the free (unlabeled) phenytoin drug in a sample with respect to specific capture antibody. A volume container filled with the fluorophore-labeled phenytoin-antibody (FA) complex leads to a high fluorescence polarization reading. Meanwhile, this fluorescence polarization is quantified as millipolarization units, or mP. With the addition of an increasing amount of unlabeled phenytoin into a solution with fluorophore-labeled phenytoin, the phenytoin-mixed solution, which revealed the competition circumstance of both labeled and unlabeled phenytoin molecules for the antibody, exhibited a low polarization reading.

In FPIA measurement, there were four samples of the phenytoin drug with concentrations of 10, 20, 30, and 40 μg/mL that were tested in DI water and 100% fetal bovine serum. Fig. 5 shows the results of the FPIA based on the competition approach. Meanwhile, a low-polarization reading resulted in a high concentration of the target phenytoin drug. In a 100% serum environment, the plot was made in an approximate parallel shift by the FPIA, leading to lower sensitivity than that in DI water.

Moreover, the experiment with three samples in concentrations of 10, 25 and 50 μg/mL in DI water was conducted by the present single free-standing piezoresistive microcantilever biosensor. In Fig. 5, the result shows the similar tendency of increasing phenytoin concentrations in DI water to that of FPIA. Despite different units used for two techniques, all signals decreased with increasing phenytoin concentrations. Likewise, a large amount of drug–antibody binding resulted in strong compressive stresses over microcantilevers that indicated a minus sign in surface stresses. The linear profile of the detection in DI water lay in a range between 10 and 80 μg/mL, and the therapeutic range of phenytoin (10–20 μg/mL) was detectable.

As a result of these profiles, the phenytoin drug detection using the present single free-standing, thermally controlled piezoresistive microcantilever biosensors showed a strong correlation with that in clinically used FPIA.

4. Conclusion

Electrical detection of small molecular phenytoin was first investigated using a single free-standing, thermally controlled piezoresistive microcantilever biosensor. Phenytoin for therapeutic drug monitoring is applied in the management of antiepileptic medication. It has been widely used with antiepileptic drugs and has a narrow therapeutic range of 10–20 μg/mL. The microcantilever sensor, micromanufactured by MEMS technique, was highly compatible with the commercial semiconductor processes. This measurement provided the phenytoin drug with the microcantilever biosensor for detection in deionized water and fetal bovine serum solutions. The detection was achieved in a linear profile of 10–80 μg/mL by the resistance changes and the associated surface stresses of the microcantilever, which covered the clinically relevant therapeutic range of the phenytoin drug. The result of the present electrically measured microcantilever biosensors showed a strong correlation of phenytoin drug detection with that in clinically used FPIA. The reproducibility and phenytoin binding affinity proved valuable in the context of antiepileptic medications using the label-free, miniaturized piezoresitive microcantilever biosensors.

Acknowledgments

The research was partially supported by the National Science Council Taiwan (NSC) under Contract no. NSC 102-2220-E-002-010. The authors also acknowledge the manufacturing help from the Nano-Electro-Mechanical-Systems (NEMS) Research Center, National Taiwan University.

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