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

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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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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

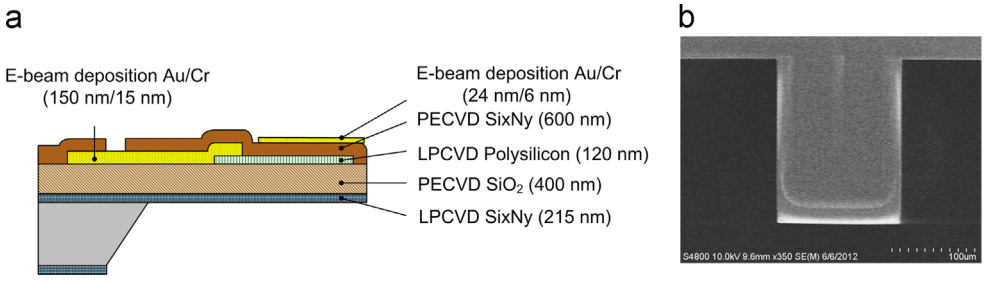

Fig. 1. (a) The piezoresistive microcantilever biosensor device and its associated process. (b) A scanning electron microscope image of a microcantilever.