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A microfluidic affinity sensor for the detection of cocaine

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ABSTRACT

We present a microfluidic aptamer-based biosensor for specific cocaine detection. The device consists primarily of a microchamber packed with aptamer-functionalized microbeads that act as a sensing surface, integrated with an on-chip heater and temperature sensor. The sensor employs a Förster resonance energy transfer (FRET) system in which a fluorophore-quencher pair of carboxyfluorescein and Dabcyl generates a signal-on response to cocaine. We demonstrate device operation by successfully detecting cocaine with a four orders of magnitude linear response in micromolar to nanomolar concentrations. The detection limit of the device is further lowered to 10 pM by concentration of a highly diluted cocaine sample, which compares well with the most sensitive detection techniques currently available. The temperature-dependent binding of aptamer–analyte complexes is then used to effect thermal release of cocaine from the sensing surface. It is found that a temperature of 37 °C can fully regenerate the sensor in pure buffer. Furthermore, testing indicated that sensor response is consistent after repeated regeneration. These results demonstrate that aptamer-based sensing on a microfluidic platform has the potential to enable low-cost, rapid, and highly specific detection of cocaine in practical applications.

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1. Introduction

The detection and quantification of trace amounts of illicit substances such as cocaine continues to be important for law enforcement and clinical medicine. Law enforcement officials must be able to detect trace amounts of cocaine in probationary offenders, and larger concentrations concealed by suspected users employing masking agents. In clinical medicine the detection of street drugs in patients of pain management practices requires frequent testing [1] that can be prohibitively expensive. Conventional methods available for cocaine detection include chromatography, presumptive testing, and immunoassays. Among these, gas chromatography–mass spectrometry (GC–MS) continues to be the gold standard in illicit material detection [2] given its sensitivity and reliability. Other chromatographic techniques, such as liquid chromatography (LC) and thin-layer chromatography (TLC) are also commonly used. In GC a sample of analyte is pushed through a column of adsorbent media, separating the constituents. LC systems are very similar, using a liquid as the carrier fluid instead of a gas, and TLC draws fluids across an adsorbent using capillary action. Unfortunately, GC–MS is inherently expensive, requiring a great deal of time, complicated equipment, and trained personnel. LC is typically even more expensive, and along with TLC gives poorer results due to its limited separation efficiency [3]. A more time-efficient alternative to chromatographic methods is presumptive testing using colorimetric reagents that rapidly react with a number of chemicals, but this approach is limited by a lack of specificity in that the reagents react non-specifically with target analytes [4,5]. To address the limitations of conventional techniques for detecting cocaine such as non-specificity and expense, the use of affinity sensing is highly attractive.

In affinity sensing, a target analyte is recognized by affinity binding with a receptor. That is, the analyte and receptor molecules join via specific hydrogen bonds, stacking of moieties, and analyte-induced receptor conformational changes which result in highly specific and reversible binding. Commonly used affinity receptors include antibodies, lectins, enzymes, and in particular, aptamers [6]. Aptamers are single stranded DNA and RNA oligonucleotides specifically selected for their binding affinity towards a specific target molecule [7]. Exposure to a target molecule induces conformational changes in aptamer structure facilitating binding that is both highly specific and reversible. Aptamers are easily chemically modified and are generated *in vitro*, resulting in no batch-to-batch variation in binding efficiency. As such, aptamers are attractive affinity receptors for cocaine detection. For instance, colorimetric sensing of cocaine was reported using aptamers conjugated to gold nanoparticles [8,9]. Additionally, UV absorption spectroscopy combined with aptamer-based solid-phase extraction was used to detect cocaine at micromolar concentrations in complex media on a large-scale liquid chromatography platform [10]. Cocaine sensing

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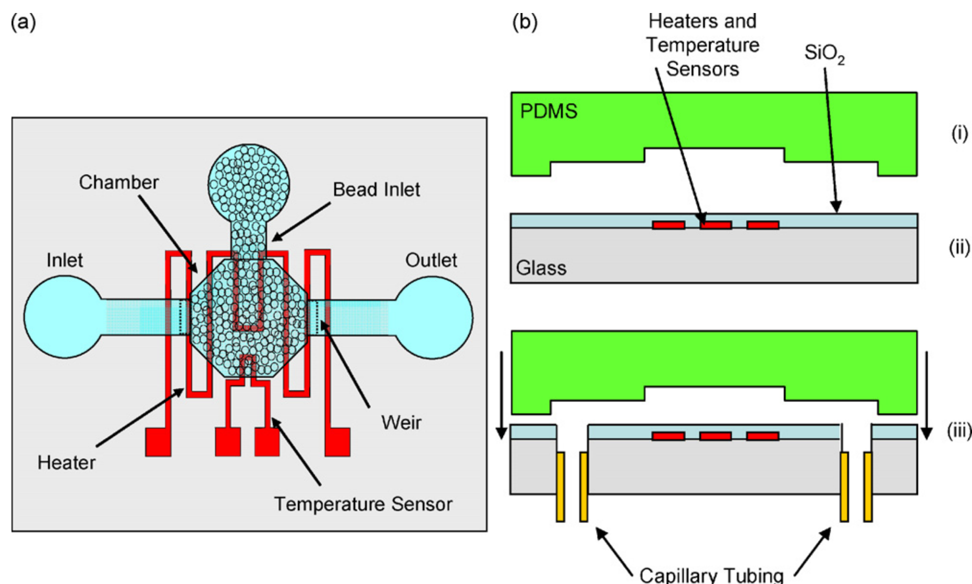

Fig. 1. (a) Schematic of the microfluidic cocaine sensor, and (b) fabrication process: (i) microfluidic features are defined in PDMS, (ii) heaters and temperature sensors are fabricated on a glass slide, (iii) the PDMS and glass slide are bonded together.

using aptamers has also been demonstrated using Raman scattering methods, in which aptamers bound to a silver colloid surface and labeled with a Raman reporter allowed detection of micromolar quantities of cocaine [11]. Furthermore, cocaine binding with a surface-immobilized aptamer can be measured via reduction of an electroactive species tethered to the distal end of the aptamer. This has allowed cocaine detection at the micromolar level using electrochemical methods [12], or with 1 nM detection limits using electrochemiluminescent methods [13]. Implemented on conventional platforms, these aptamer-based cocaine sensing approaches generally involve large reagent consumption, require bulky equipment, and are labor-intensive. These limitations hinder the realization of cocaine detection in portable, user-friendly systems.

Miniaturization technology holds potential for rapid, sensitive detection of cocaine with minute sample quantities. A microfluidic adaptation of a standard presumptive test for cocaine was fabricated, consisting of a microchannel into which was deposited a small amount of cobalt thiocyanate [14]. While still limited by conventional presumptive testing, the microfluidic device led to reduced sample consumption and allowed for device regeneration. In a different approach, a microfluidic sensor based on antibodies attached to a quartz crystal microbalance demonstrated rapid detection (<1 min) of nanograms of cocaine [15]. However, the use of antibodies makes the sensor regeneration difficult, and may also lead to batch-to-batch variations in sensor performance. More recently, a microfluidic continuous-flow aptasensor was reported that uses an electrochemical method for the detection of cocaine among other compounds in blood serum-based samples [16]. Micromolar detection limits were demonstrated.

This paper presents a microfluidic aptamer-based biosensor for cocaine that uses a cocaine-specific DNA aptamer modified with a fluorophore. Based on a device architecture designed for highly specific solid-phase extraction [17,18], this sensor is capable of low-cost quantitative cocaine detection in a highly specific, signal-on, and label-free manner. We demonstrate that the device is capable of specifically detecting cocaine at concentrations as low as 10 pM, with a linear response over four orders of magnitude. Additionally, the device can be conveniently regenerated for reuse by a modest temperature change via on-chip temperature control. As such, the device can potentially be used for sensitive and rapid detection of cocaine in practical clinical and law enforcement applications.

2. Principle and design

The device consists of a microfluidic chamber and channels fabricated from (poly)dimethylsiloxane (PDMS) and bonded to a glass substrate integrated with a resistive micro-heater and temperature sensor (Fig. 1a). The inlet and outlet microchannels are utilized for sample and buffer introduction and removal from the device, while an auxiliary microchannel is used for bead packing. Microweirs (15 μm high) separate the microchamber (130 μm high) from the inlet and outlet microchannels and serve to retain the beads in the microchamber. The on-chip microheater has a serpentine layout and directly covers the microchamber area to facilitate uniform heating of the microchamber area. A resistive temperature sensor is placed at the center of the microchamber to provide accurate temperature measurement for thermal control. The device was fabricated using soft lithography and lift-off techniques (Fig. 1b). A mold was fabricated from the negative photoresist SU-8, and a 10:1 mixture of PDMS pre-polymer and curing agent was cast over the mold to fabricate microchannels in a PDMS slab. This was bonded to a glass slide with integrated heaters and sensors. Details of the fabrication process are described elsewhere [18].

A Förster resonance energy transfer (FRET) system is utilized to signal cocaine binding to the aptamer. FRET systems involve the coupling of a fluorescent molecule that emits visible light (fluorophore) to another fluorescent molecule that absorbs visible light and emits at invisible wavelengths (quencher). We use carboxyfluorescein (FAM) as a visible-range fluorophore, which, attached to a cocaine-specific aptamer (below), is characterized by a peak absorption wavelength of 494 nm and a peak emission wavelength of 518 nm [19]. Dabcyl is employed as a quencher molecule, which absorbs light over a wide range of wavelengths and dissipates the light as infrared energy. When placed in close proximity to FAM, Dabcyl causes a drastic reduction in visible emission during excitation at 494 nm. To exploit the FRET system within the microfluidic device for aptamer-based cocaine detection, we employ a DNA aptamer that is adapted from the sequences originally obtained for free solution aptamer–cocaine binding [20]. A FAM molecule is attached to the 5′ end of the aptamer, and a biotin molecule at its 3′ end. This allows the aptamer to be immobilized onto a streptavidin functionalized surface via biotin–streptavidin interaction with the FAM molecule at the free end (Fig. 2a). In order to establish a baseline signal, a Dabcyl molecule, attached to the

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