A MICROFLUIDIC AFFINITY APTASENSOR

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ABSTRACT

We present a prototype biosensor suitable for selective detection of analytes and controlled release of metabolic drug molecules. The device consists of microfabricated PDMS channels on glass with two microchambers (sensing and delivery) filled with ribonucleic acid aptamer responsive media. Using adenosine monophosphate (AMP) as an analyte and drug molecule, we demonstrate detection of trace amounts of AMP as well as controlled delivery of AMP by thermal activation. The system is viable and regeneratable in aqueous medium, providing long-term functionality on a biocompatible platform.

Keywords: Aptamer, aptasensor, drug delivery, point-of-care

1. INTRODUCTION

Modern warfare demands reliable point-of-care (POC) devices. Self-diagnoses and medical treatment under extreme duress is difficult in the field. Hence, POC chips require unambiguous sensing, stable drug delivery, and simplicity. Given such conditions, POC instruments need selective and responsive analyte recognition surfaces.

Biosensors are increasingly important components in POC chips. Antibody- and enzyme-based sensors are most common, but have limitations. Enzyme sensors require auxiliary reagents and/or separation steps [1], while antibody sensors exhibit limited shelf life and labelling degradation. Aptameric sensors based on RNA and DNA can alleviate these problems. They can be engineered for bio-specificity toward possibly any target molecule that binds nucleic acid. In addition, aptamers offer room temperature stability, easy immobilization to surfaces and controlled functionality [2].

Microfuidic POC chips may also administer drugs utilizing a responsive surface. Existing functional surfaces involve hydrophobic and ion-exchange media which lack high selectivity to target molecules [3]. Thus, unwanted impurities are retained with the target drug which degrades device performance. Unlike these former retention mechanisms, the selectivity of aptamers insures controlled retention and delivery of only the desired drug.

There are applications which may benefit from integrating biosensing and drug delivery on a single device. One example is the diagnosis and treatment of shock (hypotension and tissue death from inadequate perfusion) caused by hemorrhage (shock) or infection (septic). Our future goal is a system that manages this condition using a POC chip that detects and administers the hormonal peptide vasopressin [4]. As a model system, we present a prototype aptameric device for trace detection and controlled delivery of a metabolic steroid, adenosine monophosphate (AMP). Aptamer selectivity is tested against non-specific analytes and drug release is performed by heat activation at 36 ^{o}C. Additionally, we demonstrate device reusability by regeneration of the aptamer in an aqueous medium.

2. EXPERIMENTAL METHODS

Biotinylated adenosine triphosphate aptamer (ATP-aptamer) is purified while AMP, cytidine, uridine, and guanosine triphosphate (C/U/G-TP) are synthesized and labeled with thiazole orange. Buffer solution is prepared from Tris-HCl, NaCl, KCl, and $MgCl_2$ in

water. Streptavidin coated agarose beads (~65 μm O.D.) provide support surfaces. A fluorescence microscope is employed for detection. Gold resistive heaters and sensors provide integrated thermal control while a syringe pump provides fluidic control.

A typical device is shown in Fig. 1a. Chambers **S** and **D** (8/4 × 0.8 × 0.3 mm) are for sensing and drug delivery, respectively. All solutions (5 μl) are introduced by channels **c1** and **c2** (height × width: 0.04 × 0.05 mm). Microbeads are packed into **S** and **D** via auxiliary side channels. Microfabrication is based on standard techniques (Fig. 1b). PDMS is poured over an SU-8 mold and cured while Cr/Au (15/150 nm) films are patterned on glass slips which are passivated by Si_xN_y. PDMS and glass are bonded after O_2 plasma treatment of the interface. Micro to macro interfaces are then produced with silica capillary tubing.

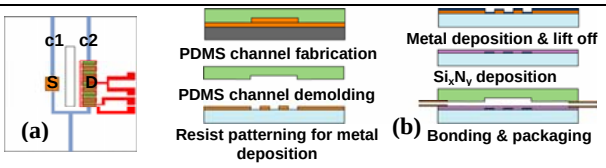


Figure 1. **(a)** Device schematic. **(b)** Chip microfabrication process flow.

The chambers are first rinsed with buffer (10 μl/min, 10 min). All following experimental washes are identical. Bio-ATP-aptamer is incubated (10 μl, 20 min) in chambers **S** and **D**. After a subsequent wash, a 10× objective is focused on a single spot in each chamber. Unless otherwise stated, AMP solution (1 μM) is introduced at 24 ^oC and 10 μl/min into the sensing and delivery chambers followed by a subsequent buffer rinse. This protocol also applies to UTP, CTP and GTP samples.

3. RESULTS

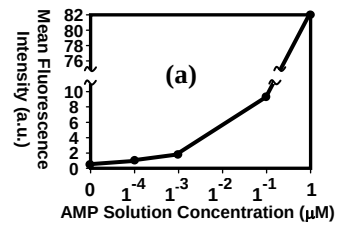
We first investigated the detection limits of the biosensor in our system. AMP is loaded in trace amounts (10^{-4}, 10^{-3}, 10^{-1}, & 1 μM; Fig. 2) into chamber **S**. Below 10^{-4} μM, no measurable signal above the background is detected. Concentrations above 10^{-4} μM however, are detectable for this device (S:N = 3:1). Additionally, this system appears to exhibit a dose responsive relationship (i.e. there is a steep linear increase in fluorescence above 0.1 μM). This may explain the detection threshold seen at the 10^{-4} μM range. Using noise analysis for this system, AMP detection on the order of 10^{-5} μM is possible with improved hardware. This is promising for practical applicability. For example, stable and shock indicating vasopressin levels in serum are 10^{-5} and 10^{-4} μM, respectively [4].

Analyte specificity is another important characteristic of the biosensor. We tested the specificity of ATP-aptamer to AMP by subjecting the sensing surface (with and without aptamer) to non-specific analytes (CTP, UTP, and GTP: 1 μM; Fig. 2b). As expected for an aptamer-free surface, all compounds produce approximately background signal. However, fluorescence is detected specifically for AMP when aptamer is present on the surface. To further emphasize the selectivity of ATP-aptamer to AMP, non-specific samples are introduced into chamber **S** directly following an AMP sample. If affinity is low, then competitive dissociation would decrease the AMP signal. As shown (Fig. 2b-iii), AMP signal is maintained which further emphasises the aptamer selectivity to target analytes.

To investigate controlled release and regenerative properties of the aptamer surface, AMP sample is retained in chamber **D** and exposed to heat (26-60 ^oC; Fig. 3a). At 36 ^oC, fluorescence decrease occurs until background levels are attained (54 ^oC). No signal implies an absence of coupled AMP/ATP-aptamer, meaning release of the drug. Hence,

adequate drug release at low temperature (36 ^{o}C) is possible. Repeatable functionality of the aptamer surface allows reusability, necessary for long-term drug treatment. To demonstrate this function, the chamber is cooled and a second AMP sample is introduced, producing a similar initial signal as the first. Implanted POC devices may require regeneration at higher temperatures (> 37 ^{o}C) which can be addressed for this system by potentially modifying the aptamer's properties.

A desirable characteristic of a drug transport mechanism is that it releases the drug completely in a controlled fashion. To generate an AMP release profile, chamber **D** is heated (36 ^{o}C) and fluorescence decrease is monitored over time (Fig. 3b). The profile suggests that AMP release is initially stalled but subsequently increases steadily until full release after 1 minute. Thus, the desired effect of a steady release profile is achieved. Presently, the kinetics of analyte release for this aptamer is not well understood. Resolving this issue can potentially enable the tuning of AMP release profiles for a given application.



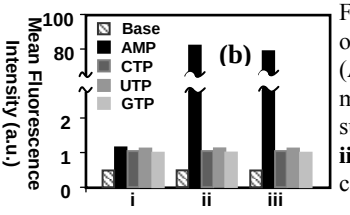
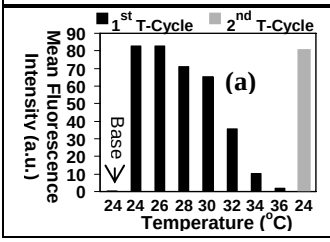


Figure 2. **(a)** Trace detection of AMP. **(b)** Selectivity of (AMP, CTP, UTP, GTP: 1 mM): **i**-non-functional surface; **ii**-functional surface; **iii**-AMP detection against competitive solution.



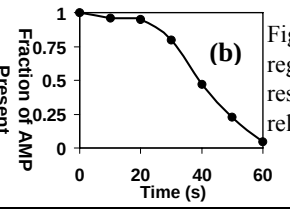


Figure 3. **(a)** Thermal release and regeneration of AMP (1 μM) from the responsive surface. **(b)** Controlled release of 1 μM sample of AMP.

4. CONCLUSIONS

We present an aptameric sensor capable of selective detection of analytes and controlled release of drug molecules. This system offers trace detection and stable delivery of AMP at 36 ^{o}C. In addition to reusability, our device is biocompatible and demonstrates a promising model for a practical shock indicating POC instrument based on vasopressin.

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