"SMART" MICROFLUIDIC PRECONCENTRATION OF SPECIFIC BIOMOLECULES

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Abstract: We present a device for selective extraction, concentration and release of metabolic molecules using ribonucleic acid (RNA) aptamers. The device consists of microfabricated PDMS channels on a glass substrate including a micro-chamber packed with an aptamer stationary phase. Features include: extraction and preconcentration of trace amounts of adenosine monophosphate (model analyte); sufficiently low temperature release and regeneration at 32.5 ^{o}C (safe for thermally sensitive target analytes and functionalized surfaces); and extensive reuse. In addition, aptamer is active in aqueous solution eliminating the required use of potentially harsh solvents and reagents.

Keywords: affinity sensor; preconcentration; purification; separation; solid-phase extraction (SPE).

1. INTRODUCTION

With widespread applications of miniaturized bioanalytical instrumentation, improvements in sample preconditioning techniques become increasingly important [1]. In particular, preconcentration (PC) is a valuable tool for improving measurement sensitivity. Existing PC systems lack high selectivity to target analytes, as desired by the needs for detecting analytes among numerous other compounds. Systems using hydrophobic [2] and ion-exchange [3] stationary phases retain not only target molecules, but unwanted analytes and impurities with similar properties. Such indiscriminate PC of target and non-target molecules makes specific detection problematic.

PC with affinity interaction resolves such issues. Antibody/lectin systems are the most employed affinity scheme. However, novel molecules such as oligopeptides, ribonucleic (RNA) and deoxyribonucleic (DNA) acid aptamers are becoming popular [4, 5]. RNA aptamers in particular, can be synthesized for possibly any target molecule that binds nucleic acid as its function [6]. In addition, they are stable at room temperature, easy to synthesize and end-chain modifiable to facilitate attachment to stationary surfaces [7, 8].

In addition to specificity, PC devices must release analytes effectively. This introduces analytes into the next analysis stage, such as mass spectroscopy for example. Release of analytes enables surface regeneration for a new sample. PC systems have used mostly harsh release methods including, changes in pH, solvents (which may denature analytes or ligands), or by photo-cleaving (which destroys the functionalized surface) [9]. An analyte release method free of these limitations is highly desirable.

We present a microfluidic device for PC and release of specific analytes. The device surfaces are functionalized with an RNA aptamer that selectively binds a target analyte. The device employs thermally induced denaturing of the aptamer for intelligent release. This occurs at 32.5 ^{o}C, a safe temperature for thermally sensitive analytes and ligands functionalizing the device surface. Since denaturing the aptamer is reversible, this permits reuse. In addition, operation is simplified as analyte capture and release occur in aqueous medium without altering solvent composition or polarity. Although applicable to a many analytes, we use a model analyte, adenosine monophosphate (AMP).

2. EXPERIMENTAL METHODS

2.1 Materials and Instrumentation

Biotinylated adenosine triphosphate aptamer (bio-ATP-40-1, or ATP-aptamer) was HPLC purified by Integrated DNA Tech. AMP was synthesized and fluorescently labeled with

thiazole orange (TO). Buffer solution (pH 7.4) was prepared from Tris-HCl (20 mM), NaCl (140 mM), KCl (5 mM), and $MgCl_2$ (5 mM) in water. Streptavidin coated polystyrene beads (50-80 µm, O.D.) were acquired from Pierce. A Nikon Eclipse TE300 microscope and CCD was employed for fluorescence detection. Temperature control was realized with a thermoelectric device and type-K thermocouple. A New Era NE-1000 syringe pump enabled flow in the device.

2.2 Device Design and Fabrication

A device schematic is shown in Fig. 1. Channels c1 and c2 (5.1 mm×400 µm×40 µm) transferred sample and discharged waste from the chamber (8.7 mm×3 mm×140 µm). Microbead packing into the chamber occurred through c3. Ports p1-p3 were 1 mm in radius and 140 µm thick. Chamber and microfluidic network volumes respectively, were 3.09 µl and 3.60 µl.

Channels were fabricated using PDMS micro-molding (Fig. 2). A mold was created on a 4-in silicon wafer by patterning SU-8. PDMS pre-polymer solution was mixed (10:1; *w*:*w*), degassed, and semi-cured (70 °C, 50 min) over the mold. In parallel, glass substrates were cleaved (25 mm×30 mm) and drilled to create ports p1-p3. The semi-cured PDMS sheet was removed from the mold, aligned, and bonded to the glass following O_2 plasma treatment of the bonding interface. Permanent bonding was realized with a final bake (25 min at 85 °C).

Packaging of the device was accomplished by inserting silica capillary and Tygon tubing, (0.6 mm ID, 0.7 mm OD) and (0.6 mm ID, 3.18 OD), respectively into ports p1-p3. The interfaces were then sealed with epoxy.

2.3 Experimental Procedure

The device was mounted on the microscope stage using clips or double-sided tape. A blue excitation filter combined with a green-pass dichroic mirror was used. A 10× objective was

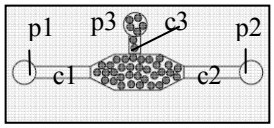

Figure 1. Schematic of the PC device.

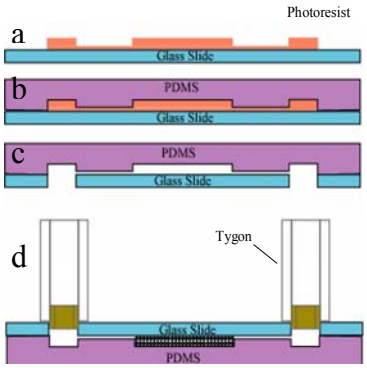

Figure 2. Fabrication process: (a-c) PDMS channel processing; (d) packaging.

kept focused on a single area of the chamber.

The chamber was initially rinsed with buffer (50 µl/min, 10 min). All following experimental rinses are identical. Streptavidin coated beads were introduced via c3 by manual pressure. The chamber and channels were rinsed and bio-ATP-aptamer was injected (20 µM, 20 µl, 10 µl/min) and incubated (20 min) in the chamber. After a final rinse, we established a fluorescence control.

Extracting distinct concentrations of AMP (24.5 °C, 10 µl, 10 µl/min) established a fluorescence intensity curve. All experiments used the above injection parameters. Solution concentrations ranged from 0.1-10 µM and fluorescence was detected after rinsing between separate extractions.

For PC of AMP, multiple solution injections were used. Two devices (*Device 1* & *Device 2*) were consecutively loaded with 200 nM and 500 nM injections, respectively. On either device each injection was incubated (5 min), rinsed, and detected for fluorescence before the next injection occurred.

3. Results and Discusion

3.1 Fluorescence Signal vs. AMP Concentration

To estimate the relationship between fluorescence signal intensity and surface concentration, AMP solution was extracted at increasing concentrations onto multiple devices. We observed a roughly linear increase in signal intensity as AMP concentration in solution increased (Fig. 3). This trend is likely caused by the AMP concentration range being well below

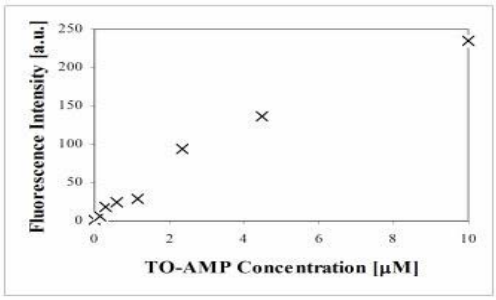

Figure 3. Fluorecence signal vs. TO-AMP concentration in solution.

the concentration threshold corresponding to the binding capacity of the bead matrix. Hence, the amount of captured molecules on surface appeared proportional to the amount in solution. This observation also implies that Fig. 3 can be used to estimate the density of captured AMP molecules on the affinity chamber surface.

3.2 Specific Preconcentration of TO-AMP

Highly specific PC is a valuable tool for preconditioning biomolecular solutions in bioanalytical applications. We tested preconcentration with 2 dilute solutions of TO-AMP on separate devices by extracting multiple injections on each device (Fig. 4). In both experiments, fluorescence signal increased after each consecutive sample load indicating increased concentration of bound TO-AMP on the surface. In addition, Device 1 and 2, after a roughly 10-fold PC, showed no sign of signal saturation within the tested injection range, meaning the surface was capable of concentrating yet more analyte. This is significant, for to attain the maximum PC factor of this device, either discrete or continuous injections will be used. The maximum device PC factor will be investigated in future research. However, Figs. 3 and 4 suggest that near 100-fold PC is possible.

According to Fig. 4, the PC trends of both experiments were quite different. *Device 1* showed a non-linear and slightly discontinuous relationship between fluorescence intensity and injection number (Fig. 4a). *Device 2* on the other hand, exhibited a proportionally increasing relationship between fluorescence intensity and injection number (Fig. 4b). We speculate that at low injection numbers, the dilute solution (200 nM) may not render sufficient coupling of AMP to aptamer to produce a consistent fluorescence signal. This would explain why at 19 injections, the specific signal (signal amplification/injection) increased for Device 1. Initially, dilute injections were presumed to produce a more linearly increasing specific signal but we observed the opposite. Future work will address this issue.

3.3 Release of Analyte and Device Regeneration

To investigate the aptamer thermal release properties, a 10 µM AMP solution was extracted and eluted for a range of temperatures (30-50 ^{o}C) (Fig. 5). After extraction of AMP on the aptamer surface, a high intensity fluorescence signal was obtained. At 32.5 ^{o}C, there was a sharp decrease in signal intensity (near baseline). As the temperature was further increased to 47.5 ^{o}C, the signal matched the baseline intensity. No signal implies an absence of coupled AMP on the device affinity surface, meaning release of analyte. Thus, our device exhibits adequate release of a captured target analyte at sufficiently low temperature (32.5 ^{o}C). Regeneration at this temperature does not endanger the viability of thermally sensitive biomolecules.

It was desired to test the functionality of the aptamer surface post-release of AMP since the streptavidin-biotin (SB) interaction was reported to denature at 70-80 ^{o}C [10]. An extraction of 10 µM AMP (*Extraction A*) was followed by elevated temperature release (75-85 ^{o}C), which was in turn followed by a second extraction (*Extraction B*) (Fig. 6). Release at 75 ^{o}C subsequently produced an *Extraction B* signal nearly equal to that of *Extraction A*, suggesting a functional aptamer surface after undergoing a 75 ^{o}C regeneration. Hence, the device was reusable. In contrast, release at 85 or 95 ^{o}C produced *Extraction B* signals significantly lower than in *Extraction A*. This could only be caused by a lack of aptamer for AMP to bind, the result of a compromised SB link. Thus, the surface functionality was destroyed. Devices with such high temperature release of analytes would be rendered useless beyond the first regeneration cycle. These results agreed with published findings. Also, since this system demonstrated sufficient release well below these values, it never endangers the functionality of its aptamer surface permitting extensive reuse.

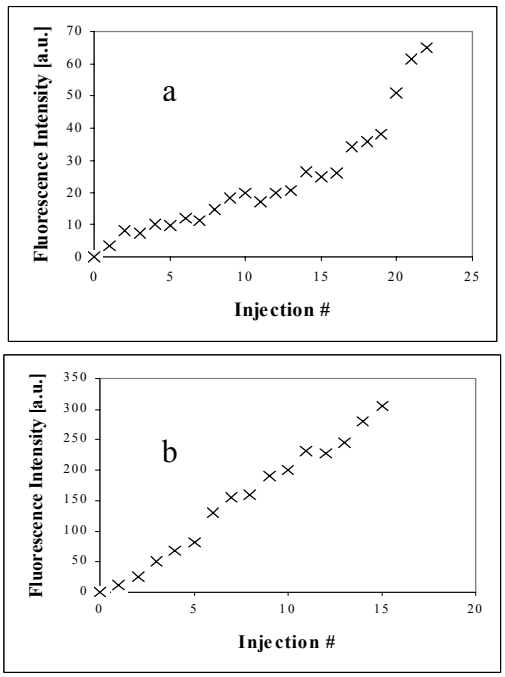

Figure 4. PC of AMP. (a) Device 1: 200 nM injections. (b) Device 2: 500 nM injections.

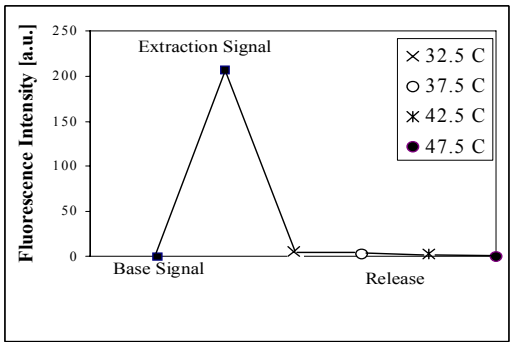

Figure 5. Thermal release of AMP from bead surfaces incubated with 10 μM injection.

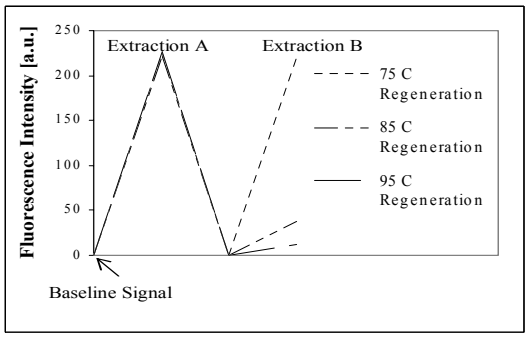

Figure 6. Attempted extraction of AMP after release at (a) 75 °C, (b) 85 °C, and (c) 95 °C.

4. CONCLUSION

Our device offers distinct advantages over current PC systems. Features include selective extraction, regeneration at 32.5 °C (safe for sensitive analytes and ligands), and simple fabrication. In addition to reusability, being functional in aqueous medium eliminates need of harsh solvents or reagents. Also, with the possibility of 100-fold PC, this device makes an attractive analysis tool in the biomedical and biochemical industries.

5. ACKNOWLEDGEMENT

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