A PDMS / PAPER HYBRID MICROFLUIDIC DEVICE INTEGRATED WITH GRAPHENE OXIDE-BASED NANO-BIOSENSORS FOR MULTIPLEXED PATHOGEN DETECTION

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ABSTRACT

We have developed a polydimethylsiloxane (PDMS) /paper hybrid microfluidic system integrated with aptamer-functionalized graphene oxide (GO) nano-biosensors for one-step multiplexed pathogen detection. The paper substrate used in this hybrid microfluidic system facilitated the integration of nano-biosensors on the chip, and avoided complicated surface treatment and aptamer probe immobilization in a PDMS or glass-only microfluidic system. *Lactobacillus acidophilus* was first used as a bacterium model to develop the microfluidic platform, with a limit of detection of 11.0 cfu/mL obtained. We have further successfully extended this method to multiplexed detection of two infectious pathogens-*Staphylococcus aureus* and *Salmonella enterica*.

KEYWORDS: PDMS/paper hybrid microfluidic device, Pathogen detection, Aptamer, Graphene oxide; Nanosensors.

INTRODUCTION

Pathogens often cause serious economic losses and public health concerns throughout the world, such as the massive outbreak of Severe Acute Respiratory Syndrome (SARS) in China in 2002-2003. Over the past decades, a variety of methods, including DNA-based methods (e.g. DNA microarray [1] and DNA sequencing technology) and enzyme-linked immunosorbent assay (ELISA), have been employed for pathogen detection. Conventional DNA-based methods, however, either depend on expensive and high-precision instruments, or require cumbersome sample treatment procedures (e.g. cell lysis and DNA purification). ELISA can be used for direct pathogen detection. Nevertheless, antibodies are more expensive and can easily become denatured and lose their activities to bind to pathogens. This paper reports a PDMS/paper hybrid microfluidic system integrated with aptamer-functionalized GO nano-biosensors for simple one-step multiplexed pathogen detection.

Numerous PDMS-based and paper-based microfluidic systems have been developed for various applications. Each device substrate has its own advantages and disadvantages. For instance, paper-based microfluidic devices provide a new low-cost platform for different applications related to health care and environmental monitoring in low-resource settings. But paper does not offer as high performance as PDMS does in liquid control. On the other side, PDMS microdevices are often associated with additional complicated chemical surface modifications for probe immobilization. Therefore, hybrid microfluidic devices that can combine advantages of both device substrates is desired, and may provide a simple solution to address the drawbacks of single substrates. However, PDMS/paper hybrid microfluidic systems that take advantages of both substrates are rarely reported. As far as we know, this paper might be the first report to present a PDMS/paper hybrid microfluidic system integrated with DNA biosensors.

EXPERIMENTAL

The hybrid microfluidic system includes two PDMS layers and one glass plate as the bottom layer, as shown in Figure 1. The top PDMS layer that has 32 micro-channels (100 μm wide and 100 μm deep), inlet reservoirs and one shared waste reservoir in the center is designed for reagent delivery. The middle PDMS layer, also called the incubation layer, has four 3 x 8 microwell (2.0 mm in diameter and 3.0 mm in depth) arrays, where incubation and detection are carried out. The total 96 wells can allow for 96 tests from one assay, thus providing high-throughput analysis. A piece of circular chromatography paper is punched into small round pieces (Φ 2.0 mm), placed inside each microwell, and served as the substrate for adsorbing the aptamer-functionalized GO in subsequent steps.

Table 1. Sequences of aptamers for pathogen sensing

Aptamers	**Sequences** (5'-3')
L. acidophilus (FALA)	cy3- ATC CGT CAC ACC TGC TCT ACG GCG CTC CCA ACA GGC CTC TCC TTA CGG CAT ATT ATG GTG TTG GCT CCC GTA T
S. aureus (FASA)	cy3- GCA ATG GTA CGG TAC TTC CTC GGC ACG TTC TCA GTA GCG CTC GCT GGT CAT CCC ACA GCT ACG TCA AAA GTG CAC GCT ACT TTG CTA A
S. enterica (FASE)	cy3-TAT GGC GGC GTC ACC CGA CGG GGA CTT GAC ATT ATG ACA G

Aptamers, oligonucleic acids or peptides molecules, can bind to a specific target molecule as 'antibodies' do. Unlike antibodies, aptamers, however, are more stable and can be easily synthesized. The DNA sequence information of the

three aptamers used for the detection of *Lactobacillus acidophilus* (*L. acidophilus*), *Staphylococcus aureus* (*S. aureus*) and *Salmonella enterica* (*S. enterica*) is listed in Table 1 [2-4].

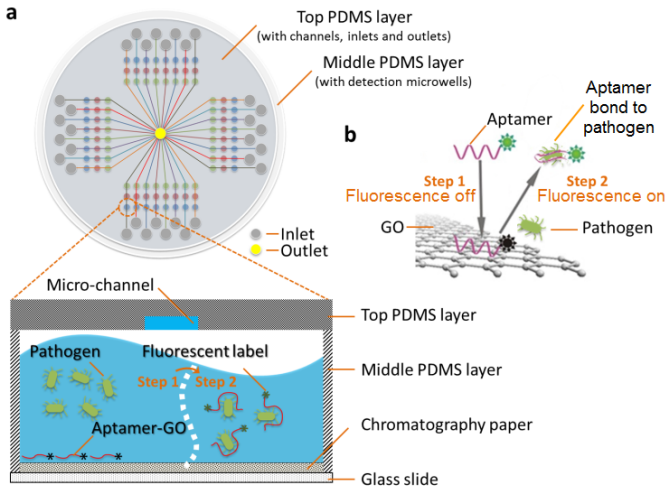

Figure 1: Schematic of the PDMS/paper hybrid microfluidic system for one-step multiplexed pathogen detection using aptamer-functionalized GO nano-biosensors. (a) Top view; Inset, Cross section view; (c) Principle of one-step "Turn-on" detection.

RESULTS AND DISCUSSION

Fig. 1c shows the main principle of the one-step pathogen detection biosensor using aptamer-functionalized GO in this hybrid microfluidic system. GO is a 2D nanomaterial with an extraordinary distance-dependent fluorescence quenching property, thus allowing GO to serve as an excellent quencher to different fluorescence dyes [5]. The fluorescence is quenched when fluorescent-labeled aptamers are adsorbed on the GO surface (Fluorescence 'OFF'). However, when the corresponding target pathogen is present, the aptamer will bind specifically to the target pathogen, resulting in the release of the aptamer from GO and reversing the quenching effect (Fluorescence 'ON'). In the absence of the target pathogen, no fluorescence restoration is detected. Instead of using complicated surface modification procedures for aptamer probe immobilization, we use a simple strategy to integrate the aptamer-functionalized GO biosensor in the microfluidic biochip through the novel use of the porous chromatography paper as a 3D storage substrate to adsorb the aptamer-GO complex in microwells. The high surface-to-volume property of the porous paper improves reaction kinetics for rapid assays. Once the paper is dry, the device is ready to use. The pathogen test only needs the one-step loading of pathogen samples into detection microwells, even without the need of an additional washing step. Therefore, this one-step 'Turn-on' mechanism offers high simplicity in pathogen detection.

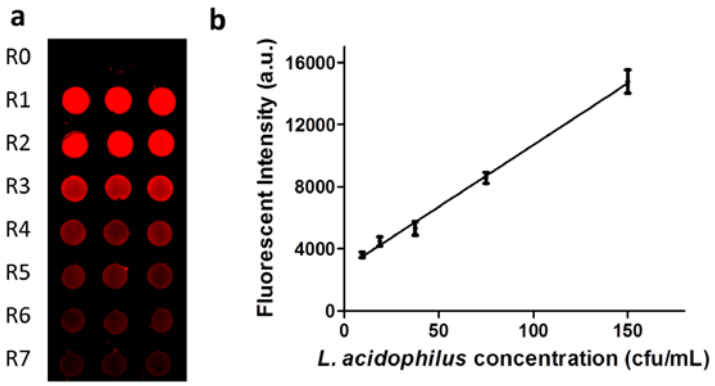

Figure 2: Detection of L. acidophilus. (a) A fluorescent image of a 3x8 well array in the detection of L. acidophilus. R0-R7 represent different concentrations of L. acidophilus from R0 (0 cfu/mL, colony-forming units per milliliter), R7 (4.7 cfu/mL) to R1 (300.0 cfu/mL). (b) Calibration curve (R^2, 99.7%).

L. acidophilus was firstly employed to develop the microfluidic system for pathogen detection. Aptamer concentration, quenching time and recovery time were optimized. After optimization (Data not shown), 1.0 μM aptamer concentration, 8 min quenching time and 10 min recovery time were used for subsequent experiments. Under optimized conditions, various concentrations of *L. acidophilus* were tested on the chip, and the results were shown in Figure 2. The fluorescent image in Figure 2a shows that with the concentration increase of *L. acidophilus*, higher fluorescence intensity was recovered. As shown in Figure 2b, the linear range of the calibration curve is from 9.4 to 150.0 cfu/mL, with a correlation coefficient of 99.7%. The limit of detection (LOD) for *L. acidophilus* was calculated to be ~11.0 cfu/mL, based on the usual 3σ.

On the basis of the one-step detection of *L. acidophilus*, we also investigated the capacity of our approach in the detection of more complex pathogen systems -- multiplexed pathogen detection. Herein, we chose two common food-borne bacterial pathogens, *S. aureus* and *S. enterica*. To validate the selectivity of the approach for *S. aureus* and *S. enterica* and void interference from each other, cross reaction was investigated by testing these two pathogens with their corresponding and non-corresponding apatmers separately. As shown in Figure 3a, when the blank, *S. aureus* and *S. enterica* samples were introduced separately from vertically-oriented channels, only microwells immobilized with corresponding aptamers to the target pathogens showed positive results, indicating high specificity of the method.

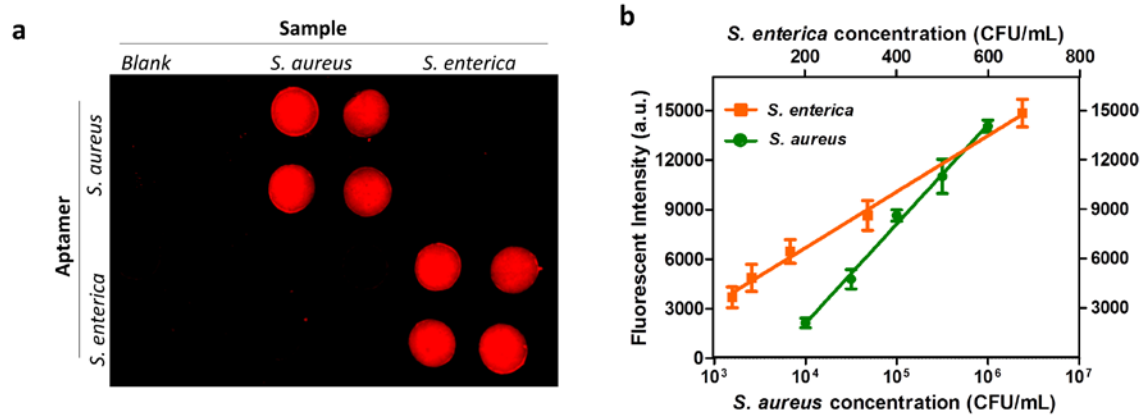

Figure 3: Multiplexed detection of S. aureus and S. enterica. (a) Cross reaction study. S. aureus, 106.0 cfu/mL; S. enterica ,1375.0 cfu/mL. (b) Calibration curves, with R^2 for S. enterica and S. aureus of 99.5% and 99.3%, respectively.

After ruling out the interference from each other, simultaneous detection of *S. aureus* and *S. enterica* was performed using the hybrid microfluidic system. As shown in Figure 3b, the linear ranges of the calibration curves were 42.2-675.0 cfu/mL for *S. enterica* and 10^4-10^6 cfu/mL for *S. aureus*. The LODs for *S. enterica* and *S. aureus* were approximated to be 61.0 and 800.0 cfu/mL, respectively. This result indicates comparable sensitivity of our microfluidic approach with some DNA amplification-based methods even without DNA extraction, amplification and purification [6].

CONCLUSION

We have developed a simple, fast and multiplexed pathogen detection method using a PDMS/paper hybrid microfluidic system integrated with GO-based nano-biosensors. Our microfluidic system has four significant features. (i) It is simple. The detection of pathogenic microorganisms requires only a one-step detection procedure, without DNA purification procedures. (ii) The PDMS/paper hybrid microfluidic system combines advantages from both substrates. (iii) The approach is fast. The assay takes only ~ 10 min from a ready-to-use microfluidic biochip. (iv) This system has significant potential in the rapid detection of a wide variety of pathogens, including plant, animal, food-borne, biodefense and other infectious diseases.

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