DEVELOPMENT OF A MICROFLUIDIC DEVICE USING NANOPARTICLE-BASED BIO-BARCODES FOR ULTRA-SENSITIVE DETECTION OF PROTEINS

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Abstract

We present a microfluidic system that allows for ultra-sensitive protein detection. The system integrates the bio-bar-code amplification (BCA) [1] approach with silver staining on a single microfluidic device to detect Prostate Specific Antigen in concentrations as low as 500 attomolar (300 proteins in the entire sample), using several orders of magnitude less sample volume than necessary for the bench-top assay.

Keywords: protein detection, Bio-Bar-Code, polyDuramide, nanoparticles

1. Introduction

Recently, a technique was developed by the Mirkin group that allows for ultra-sensitive detection of proteins via the combined use of magnetic microparticles and gold nanoparticles. The magnetic particles (MMP) are functionalized with monoclonal antibodies that specifically recognize a target protein. Gold nanoparticles (NP) are functionalized with polyclonal antibodies, and are also densely coated with a distinctive DNA sequence (bar-code DNA) unique to the target. After binding and recognition, the sandwich assay is magnetically separated, followed by thermal release of the target DNA and detection, including in some cases a target amplification step by PCR. Prostate-specific antigen (PSA), a potential marker for prostate and breast cancer, was detected by this approach down to a limit of 30 attomoles, several orders of magnitude below current clinical methods.

In new work, we have demonstrated this technique in a microfluidic device, using an integrated in-line detection scheme in which separation, purification and detection of PSA from upper attomolar to lower femtomolar has been accomplished. Several iterations of protocol development were performed to adapt the standard BCA approach to a microchannel format.

2. Theory

Microfluidic chips (3.2cm long and 1.5cm wide) were fabricated using a combination of polydimethylsiloxane (PDMS) and glass slides. Multilayer soft lithography was employed to provide individually addressable micromechanical valves that are controlled by pressure sources [2]. Pressure sources were actuated by a National Instruments DAQ card and a graphic interface developed with Labview 6.0.

It is well known that protein adsorption occurs on both glass and PDMS surfaces. Experiments conducted using blank samples and samples containing 3 nM PSA in the microfluidic chips with and without a novel hydrophilic coating (poly-N-hydroxyethylacrylamide, or polyDuramide) revealed that the coating significantly reduces the adsorptive interactions of both the target protein and bar-code DNA coated NP with the glass and PDMS surfaces [3], thereby reducing background noise and increasing signal specificity. The results of these experiments are shown in Figure 1.
Initial devices used off-chip PCR to verify the release of bar-code DNA product in the presence of PSA. Next-generation devices include a bar-code DNA capture and detection area for on-chip silver staining, thus completing the design of a fully integrated device as seen in Figures 2 and 3. “Capture DNA,” specific to half the bar-code DNA sequence, is spotted on the glass surface of the chip to allow capture of the bar-code DNA.

3. Experimental

In a typical experiment, the protocol is divided into two steps: target separation and bar-code detection. Before the sample is introduced to the chip, the separation area is coated with a polyDuramide solution. The target protein is then mixed with MMP and NP probes and incubated for 30 min at 37 °C to allow the protein to become sandwiched between the probes. The sample is then loaded into the separation area on the chip and the sandwiched protein is removed from the sample through application of a magnet underneath the chip. The supernatant is removed with a PBS buffer wash while the magnetic parts are still immobilized on the chip surface. Next, the bar-code DNA is released from the NP by flowing deionized water through the chip. The bar-code DNA hybridizes with capture DNA strands that are patterned in the detection area.

Bar-code detection is carried out by flowing through a second set of NP functionalized to match the other half of the bar-code DNA sequence to complete the sandwich assay. PBS buffer is run through to remove any unbound NP. Next, a surfactant solution is drawn through the detection area to prime the NP. Finally premixed silver staining solution is added, which preferentially stains the gold nanoparticles. The top PDMS piece is then removed and the results are visualized.

4. Results and discussion

The chips are disposable, although the top polymer part can be reused several times with only the DNA coated glass slide being replaced. Results from the employed protocol can be visualized by simple inspection, if for example the system is used in field operations. Quantitative data (Figure 4) was obtained from the microfluidic chips using a Verigene ID optical detection instrument that measures scattered light (Nanosphere, Inc., Northbrook, Illinois). The data indicates that this system can clearly resolve protein concentrations in the femtomolar level and shows that even lower concentrations can be detected in the future.

5. Conclusions

We have presented an integrated microfluidic chip that sequentially processes microliter volumes to detect variable concentrations of scarce protein analytes. PSA levels as low as 500 attomolar were detected. Future work includes parallel sample screening as well as detection of multiple protein targets.

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References

Figure 1. PolyDuramide testing results.

Figure 2. Microfluidic Device.

Figure 3. Schematic diagram of a microfluidic chip. Light gray depicts control valves. Dark gray indicates fluid channels. Alignment marks are used for proper orientation of control valves with fluidic channels during fabrication.

Figure 4. Optical scanning of developed silver stained biobarcode DNA. The control experiment (left) was run with no PSA present while 500 aM and 50 fM results are presented center and right. Average signal intensities are given as 2.2, 4.9 and 12 respectively.