A Microfluidic Chip for Bio-Bar-Code-Based Detection of Proteins

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An ultrasensitive nanoparticle-based biomolecule detection method, known as Bio-Bar-Coding (BBC) was recently developed by the Mirkin Group, which utilizes magnetic microparticle probes (MMP) functionalized with monoclonal antibodies that specifically bind to the target of interest [prostate-specific antigen (PSA) in this case; PSA is a prostate cancer marker and a potential breast cancer marker] and gold nanoparticle probes (NP) coated with both polyclonal antibodies and a distinctive DNA sequence (bar-code DNA), which sandwich the target bound to the MMP[1,2]. We demonstrate this technique in a single microfluidic device that integrates magnetic separation of the captured target, release of a number of bar-code DNA strands for every target recognition event, and the subsequent detection of the bar-code DNA using a chip-based DNA detection method. PSA concentrations as low as 500 attomolar (~200 target proteins in entire sample and this is four orders of magnitude more sensitive than current PSA assays), using only 1 micoliter total sample volume, were detected in this manner on a single chip.

The BBC assay was implemented as the detection method because of its 1) high sensitivity, 2) ease of adaptability to numerous different targets, and 3) straight forward realization onto a microfluidic platform. The high sensitivity of the method lends itself to microfluidics in that it allows ultra-low concentration levels to be detected in conjunction with small sample volumes without the need for enzymatic amplification, such as the polymerase chain reaction (PCR). The BBC assay is the only PCR-less detection method known to provide sensitivity at these low protein concentration levels. Moreover, the BBC assay can be extended to incorporate other targets in addition to PSA. Finally, the linearity of the process allows for easy automation.

In a typical experiment, the protocol can be divided into two steps: target protein separation and bar-code DNA capture. For target protein separation, the target protein is sandwiched between MMP functionalized with target specific antibodies and NP that are coated with a unique target DNA sequence and a second target specific antibody. This is accomplished by premixing the target sample with MMP and NP probes and loading an aliquot onto the chip. The sandwiched targets are then removed from the sample through application of a magnetic field. The sandwiched complexes are then immobilized on the bottom of the chip, thus permitting wash steps to be carried out to remove all of the unreacted components from the sample. Next, the bar-code DNA strands are released from the NP by flowing water over the sandwiched target and hybridized with complementary DNA strands that are

patterned in the detection part of the chip prior to the experiment. For the bar-code DNA capture step, a second set of the oligonucleotide-modified NPs is then drawn through the detection area of the chip to hybridize bar-code DNA between the capture DNA on the chip and bar-code DNA complement modified NP. Finally, the immobilized NP signal is amplified using silver staining, which is catalyzed by the presence of NPs on the chip[3].

Microfluidic chips are fabricated using a combination of polydimethylsiloxane (PDMS) and glass slides. Multilayer soft lithography is employed on the chips to provide micromechanical valves for directional control flow of fluids[4].

It has been well established in the literature that protein and DNA adsorption occurs on both glass and PDMS surfaces. Application of a hydrophilic coating to the separation area of the chip (N-polyhydroxyethylacrylamide or polyDuramide) proved to be effective in the reduction of the adsorptive interactions of the target protein with the glass and PDMS surfaces, thereby reducing background noise and increasing signal specificity[5,6].

Experiments were carried out with varying concentrations of PSA: negative control, 500 attomolar PSA, 5 femtomolar PSA, and 50 femtomolar PSA. Quantifiable data was obtained by using an optical detection instrument to scan the stained microfluidic chips. A linear relationship was observed between the signal intensity and protein concentration over the entire testing range. In our second generation chip, we developed a design that allowed us to test multiple samples in parallel.

We present a microfluidic chip that sequentially processes microliter volumes to detect variable concentrations of protein analytes. This approach illustrates the integration of several key steps in a sophisticated medical application, which greatly reduces labor requirements, increases reproducibility, minimizes errors, and surpasses currently available clinical diagnostic techniques. Current work involves the development of a third generation chip that permits the screening of multiple targets.

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