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RESEARCH PAPER

A Microfluidic Microbeads Array Chip Integrated with Micro-fluid Driven Micro-pump for Discrimination of Gene Mutation

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Abstract: A novel approach for single-nucleotide detection based on the micropump-integrated microfluidic microbeads array chip and an apyrase-mediated primer extension process was developed. In this method, an integrated chip was constructed by a microfluidic chip, a primers-modified microbeads array and a micro-pump driven by evaporation and capillary effect. The target DNA flowed across the fabricated microfluidic beads array and hybridized with immobilized primer sequences. When the 3' terminus of primer matched with the target DNA in the single-base mutation site of interest, under synergistic effect of apyrase and exonuclease-deficient Klenow DNA polymerase, the matched primer extended along the template DNA sequence and incorporated the biotin-dCTP into the extended primers and immobilized it onto the surface of microbeads. Then, the streptavidin-labeled quantum dots bond with deposited biotin moities of biotin-dCTP and generated a fluorescence signal. On the contrary, there is no signal when signal-base mismatched duplexes were present in the 3' terminus of the primer. The limit of detection is 0.2 pM target DNA ($S/N > 3$) for micro-pump driven chip and 0.5 pM target DNA for liquid pressure driven chip respectively. The chip-based signal enhancement for single-nucleotide discrimination using micro-pump integrated microfluidic chip resulted in 500 times higher sensitivity than that of an off-chip test. Since the off-chip assay only detected 0.1 nM target DNA. The fluorescence signals are linear in the target DNA concentration ranging 0.5 pM to 30 pM. This method was also used to detect two multi-drug resistance gene 1 (MDR1)-associated SNP sites (C3435T and G2677T) from a human genomic sample. The fluorescence signals indicated the subject used here possessed both MDR1 3435CT and MDR1 2677TT genotypes, which were consistent with the results by DNA sequencing. This approach displays good specificity, sensitivity and stability for discrimination of gene mutation.

Key Words: Micropump; Microfluidic beads array; Apyrase; Gene mutation; Quantum dot

1 Introduction

Since the single nucleotide polymorphism (SNP) was proposed by E. Lander in 1996, it has been studied and applied extensively[1–3]. The study on the SNP revealed that single-base differences in DNA sequences are significant among different race, populations and individuals, which brought revolutionary changes in diagnosis, prevention and treatment of diseases.

At present, the main methods for gene mutation analysis include allele-specific oligonucleotide (ASO)[4], allele-specific primer extension[5], oligonucleotide ligation assay[6], and allele-specific PCR[7], etc. These methods have intrinsic shortcomings such as low specificity and high cost of analysis, which have limited the commercial applications of these methods in high-throughput detection of single-nucleotide polymorphism. Apyrase mediated allele-specific extension is recently developed for high throughput SNP analysis and

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displays several remarkable merits such as high specificity and low cost[8]. These features make it suitable for technology commercialization.

Microfluidic technology can miniaturize and integrate the basic operation steps of sample treatment such as preparation, separation and reactions and so on in biological, chemical, and medical analysis, which makes the detection process fast, efficient with small amount of sample. Due to the great potential in the fields of biology, chemistry and medicine, microfluidic technology has become an important multi-interdisciplinary subject[9–12]. The microfluidic beads sensing is a novel multiplexed microfluidic technology that integrates efficient sample handling of microfluidics, high-throughput parallel analysis of microarray, and heterogeneous recognition ability of microbead. This platform has displayed a strong academic value in the field of new analysis techniques[13–15]. However, most of the driven modes used in the platform are gravity driven or pressure driven, which makes the flow rate not easy to be controlled and thus the stability of detection is weak.

The driven and control of microfluidics are the key technology in the research of microfluidic chip, and the drive pumps used for the microfluidics is divided into two main types: mechanical micro-pump[16,17] and non-mechanical micro-pump[18,19]. A variety of powers such as electrostatic drive, steam drive and so on are used for the driven mode for the mechanical pump. However, the manufacturing process of these micro-pumps is complicate and often pulsation occurs in the liquid flow. On the contrary, there is no pulsation flow in non-mechanical micro-pump (mostly the field induced micro-pump), which can control the velocity changes ranging from a few nanoliters per minute to several hundred microliters per minute. These micro-pumps are widely applied in microfluidic systems, however, these micro-pumps need larger drive and are inconvenient to use. Recently, a novel micro-pump driven by evaporation and capillary effect has been designed to drive flow in micro-channel[20]. The integration of the micro-pump in the micro-system has demonstrated many advantages including simpler operation, low cost, small volume, flow stability, long-time use and easy adjustment of velocity and so on. These characteristics make the micro- pump good application value and potential in microsystem field.

In this study, a novel microfluidic chip that integrated the microfluidic beads array chip and micro-pump based on evaporation and capillary effect had been developed. This chip integrated efficient sample handling of microfluidics, high-throughput parallel analysis of microarray, heterogeneous recognition ability of microbead, and had the properties of adjustable and stable velocity control of micro-pump. The apyrase-mediated allele-specific extension with quantum dot labels also had been successfully applied in the developed microfluidic chip for single-base detection, and many merits had been displayed in this microfluidic bead-based enzymatic primer extension approach such as high sensitivity and specificity, maneuverable high-throughput and microanalysis properties, and good testing stability.

2 Experimental

2.1 Instruments and reagents

Polydimethylsiloxane (PDMS) prepolymer and curing agent used for fabrication of microfluidic chip were purchased from Dow Corning Corporation (USA). SuperAvidin^{TM} coated polystyrene beads (15 μm) were purchased from Bangs Laboratories (USA) and streptavidin-conjugated quantum dot was purchased from Wuhan Jiayuan Quantum Dots Co., LTD (China). The apyrase and single strand binding protein (SSB) were purchased from Sigma-Aldrich China Corporation (China). HPLC-purified oligonucleotide probes with various modifications, PCR primers, DNA Ex Taq hot start polymerase, dNTPs mixture, dATP, dTTP, dGTP, blood genomic DNA extraction kit and DNA fragment purification kit were obtained from TaKaRa Biotechnology, Inc. (China). Biotin-14-dCTP was purchased from Invitrogen Beijing Office (China). Exonuclease-deficient (Exo$^-$) Klenow DNA polymerase was obtained from the Fermentas china corporation (China). Other reagents were analytically pure, and the water used in the experiments is ultra pure water of 18 MΩ cm.

2.2 Methods

2.2.1 Fabrication of microfluidic beads array chip integrated with micro-pump

The chip was prepared based on the photoetching technology from photosensitive circuit board (PCB)[21]. First, the layout of microfluidic networks was designed by common drawing software CorelDRAW 9.0 and printed on Kodak 2400 dpi films as photomask. The photomask was covered by the positive photoresist coated. The printed circuit board (PCB) was exposed to UV irradiation for 110 s, and the exposed PCB board was incubated with 100 mL mixture of developer and water (1:20, *V/V*) for 6 min to dissolve the exposed photoresist. Second, the developed PCB plate was put into 200 mL etching solution (prepared by $FeCl_3$•$6H_2O$ and water, 1:2, *w/V*) for another 0 to 60 min incubation to etch the unprotected copper layer with an etching depth of 0–35 μm. Final, remnant photoresist on PCB board was washed away by acetone. A metal substrate (10 mm × 10 mm × 1.5 mm in width, length and depth) was needed to make an evaporation and capillary driven micro pump. The metal substrate was attached to the site where the micro-pump located on the chip master. PDMS prepolymer and the curing agent in the proportion of 10:1