APLICATION OF BIOCHIP USING THE MOLECULAR BEACON PROBE IN BREAST CANCER GENE P53 DETECTION

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1 General Introduction

Today, breast cancer remains a worldwide public health concern and about 180,000 women are diagnosed with the disease yearly in the US (Kelsey, 1993). p53, a breast cancer susceptibility gene, was first identified in 1994. People carrying a mutation (abnormality) in this gene are at an increased risk of breast or ovarian cancer. At least 10% of observed breast cancer cases in the general population are related to the genetic predisposition (Tsourkas et al. 2003). The detection of p53 offers an opportunity to characterize the function of genetic features in breast and ovarian cancer and to screen breast or ovarian cancer patients for the presence of germline mutations. Discovery of a mutation in patients can greatly effect the prediction of cancer risk and help the doctors and patient to take the appropriate steps for treatments (Chen 2000).

One of the most unambiguous and well-known molecular recognition events is the hybridization of a nucleic acid to its complementary target. A molecular beacon (MB), a short oligonucleotide with a loop and stem structure, uses this recognition feature. The stem part contains five to seven base pairs, which are complementary to each other but unrelated to the target oligonucleotide. The loop section of a MB is complementary to its target oligonucleotide (Stokes et al. 2001). A fluorescing and quenching chemical moiety is covalently attached to the end of each stem. Because the stem keeps these two moieties together in close proximity, the fluorogenic probe is unable to fluoresce. This is due to fluorescence quenching caused by the proximity between the quencher and acceptor (Marras et al., 2002). When a MB is hybridized with its complementary target, the stemis forced apart, thus resulting in the restoration of fluorescence.

In this study, we investigate the use of MB probes along with a miniaturized detection biochip system for the detection of p53 gene in solution. Previously, we have developed an integrated circuit (IC) chip, known as the multi-functional biochip (MFB), that has demonstrated great potential for field use. The MFB has a number of distinct advantages over alternate biosensing technologies (Vo-Dinh, 1988; Vo-Dinh et al., 1999; Vo-Dinh and Cullum, 2000; Stokes et al., 2001). These advantages include a fabrication process based on complementary metal oxide semiconductor (CMOS) technology and multi-analyte detection. For example, the CMOS fabrication process, allows for application specific circuitry (i.e. signal amplification and filtering) to be integrated into the chip, thereby sig nificantly reducing the size and power requirements of the system. Another important consideration is that the CMOS process is very cost-effective, which is ideal when large numbers of portable detection devices are needed. Furthermore, the chip is composed of an array of individual detector elements, each of which

could be devoted to the detection of a different biological agent for multiplexed detection. For example, in this work, a 4 × 4 array of photo-sensors was used, which could be capable of performing 16 simultaneous bio-analyses in a single, compact unit.

2 Methodology

2.1. Molecular beacons and target genes

Molecular beacon (3'-DABCYL-GGA T (Biotin dT) CG GCG CGC TTT GTA GGA TTC GTT CGA TCC-Cy5-5') and its complementary single-stranded DNA p53) (5'-CGC GCG AAA CAT CCT AAG CAA -3') were synthesized by Gene link Inc. (Hawthorne, NY) and used without further purification. The structure of this MB is Cy5 was used as the fluorophore and (4-dimethylaminophenylazo) benzoyl (DABCYL) was attached as the quencher. Because the MB was originally designed for surface immobilization, biotin was linked to the quencher end of the stem of the MB.

2.2. Hybridization

Hybridization buffer (TE) contained 20mMTris–HCl and 100mM $MgCl_2$ at pH 7.5. The required concentration of p53 gene was diluted fromitsmore concentrated solution into the hybridization solution. The purchased MB was dissolved in TE buffer and diluted using the same buffer for the experiments. In the studies of $MgCl_2$ concentration effect on fluorescence yield, the concentration of $MgCl_2$ was varied while keeping the concentration for the rest of the components in the hybridization solution constant. Hybridization was performed in PDMS wells after mixing the MB solution and the p53 gene prepared in the hybridization solution. The reported concentrations of the MB and p53 gene are the final concentration after mixing the solutions in the wells. The volume of each well is estimated as 1 µl. The final concentration for MB was 2.5 µM and for p53 gene was 0.2, 0.4, 2.0, and 5.0 µM. Following completion of the hybridization process, the readings were performed. Herring sperm (HS) DNA was denatured into single strains by boiling for 10min prior to mixing with the MB.

2.3. Biochip detection system

This detection system features an integrated circuit-based 4×4 array detector, in which each photodiode operates independently. The individual photodiodes of the 4×4 array are sensors with 900 µm ×900 µm dimensions, and each of them is arranged with 1mm center-to-center spacing. They are integrated along with amplifiers, discrim- inators, and logic circuitry on a single solid-state circuit.

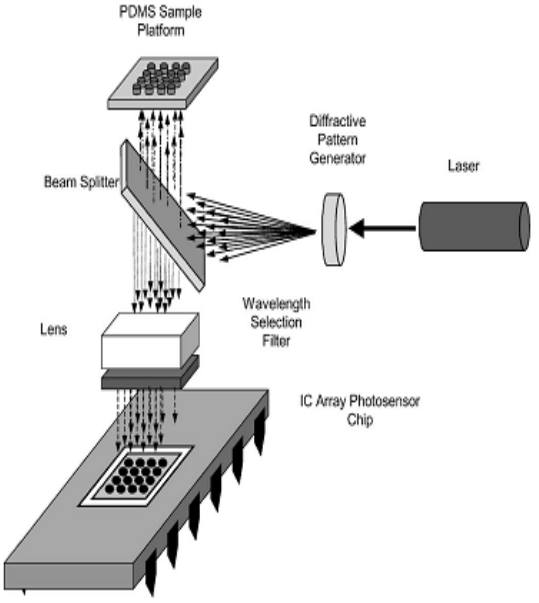

Fig. 1 Schematic diagram of the miniature biochip detection system.

The detection system consists of an excitation source, excitation and collection optics, and IC biochip. A diode laser with 5mW output power and 635 nm wavelength (Model VHK 4.9mW, Edmund Scientific) is selected for excitation of the Cy5 labels. The laser beam is launched through a diffractive pattern generator, which produced a 4 × 4 array of laser beamlets of equal intensity. The intensity of one single laser spot is estimated as ▫ 0.2mW. A molded micro well 4 × 4 plate of PDMS is visually aligned with the focused laser excitation spots. The image of the laser spot array is projected from the molded PDMS 4 × 4 microwell plate onto the corresponding 4 × 4 array of photo-sensors of the IC detector via a 2.5 cm. diameter, f/2 lens and

an emission band-pass filter (HQ 700/75 nm, Chroma Technology Corp.). The output from the IC biochip is recorded as a voltage signal by means of a digital multi-meter. A depiction of the biochip detection system is seen in Fig. 1.

3. RESULTS AND DISCUSSION

Tsourkas et al. (2003) demonstrated that the performance of a MB could be very sensitive to its structural characteristics such as probe and stem lengths. They reported that astem of at least four bases was required for lowering back-ground noise, and the shorter probe domains (22–25 bases) were required for higher selectivity. In addition, Marras et al. (2002) studied several dyes and molecules as fluorophores and quenchers. These two studies were taken as a reference point for designing the MB used in this study. The stem was composed of seven base pairs and the probe was composed of 22 bases. In order to achieve a full hybridization with the MB, a p53 gene fragment composed of 123 bases was used.

The MB probe was complementary to the 22 bases in the middle of the p53 gene. When designing the MB probe, the requirements for instrumentation were also taken into account. Because a diode laser with 635 nm wavelength was used, a Cy5 label, which absorbs at the laser exaction wavelength, was chosen.The experimental conditions were first optimized to achieve the highest fluorescence signal. Because fluores- cence signal is directly related to hybridization efficiency, the first priority was to optimize the hybridization conditions. In solution, single-stranded DNA carries negative charge. The presence of a cation in the media can accelerate the hybridization process by neutralizing (at least partially) the negative charge on the single-stranded DNA. The addition of divalent cations in the hybridization solution was reported to be the best choice (Tyagi 1996). Thus, the effect of varying concentrations of $MgCl_2$ solution on the hybridization efficiency was examined. Fig. 2 shows that a higher fluorescence yield was obtained with increasing $MgCl_2$ concentration up to 100mM $MgCl_2$, at which con- centration a plateau in fluorescence signal was attained.

A further increase in concentration of $MgCl_2$ had little effect on the fluorescence yield. A 100mM $MgCl_2$ solution was used for the following experiments reported here. Fig. 3 shows the evaluation of the system using the PDMS-well platform. The final MB concentration was 2.5 µM in all the wells. From left to right, the first four wells of the PDMS platform did not contain any solution (i.e. designated blank). The second row only contained MB

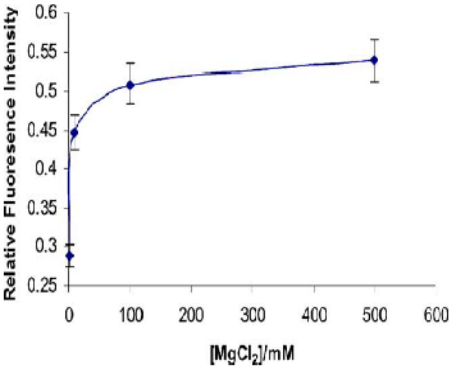

Fig. 2 Graph showing the relationship between the time a voltage generated.

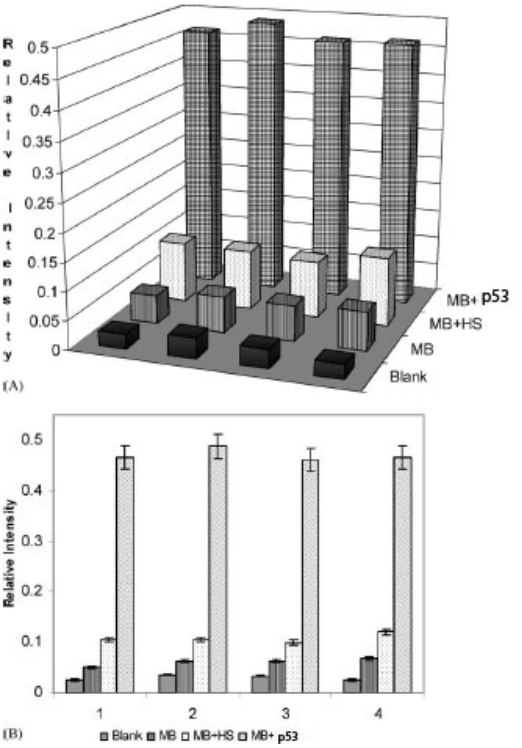

Fig. 2 (A) Demonstration of detection of p53 gene with MB probe system and comparison of with MB probe (B) Two-dimensional plot of the results and error bars of the biochip detection system.

5. CONCLUSION

The detection system and hybridization conditions were optimized for the LOD determination. It was found that the $MgCl_2$ concentration of 100mM was adequate to achieve the optimum hybridization conditions. The LOD was estimated to be 70 nM. Although 70 nM are reasonably a low amount, this detection limit needs to be improved further. Background noise due to incomplete quenching of the fluorescing component of the MB and nonspecific interaction with noncomplementary DNA sequences is a fundamental problem with MB detection systems.

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