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A new method of preparing fiber-optic DNA biosensor and its array for gene detection

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Abstract A new method of preparing fiber-optic DNA biosensor and its array for the simultaneous detection of multiple genes is described. The optical fibers were first treated with poly-l-lysine, and then were made into fiber-optic DNA biosensors by adsorbing and immobilizing the oligonucleotide probe on its end. By assembling the fiber-optic DNA biosensors in a bundle in which each fiber carried a different DNA probe, the fiber-optic DNA biosensor array was well prepared. Hybridization of fluorescent- labeled cDNA of *p53* gene, *N-ras* gene and *Rb1* gene to the DNA array was monitored by CCD camera. A good result was achieved.

Keywords: **fiber-optic DNA biosensor, DNA array, gene detection.**

Following the progress of Human Genome Program (HGP), more and more giant DNA sequence information has been produced, and how to use this genome information has been a hot issue in life science. The key problem is to develop some new methods to process the information efficiently. It is in this situation that DNA chips have been invented.

All kinds of DNA chips are based on the principle of nucleic acid hybridization, which is one of the central tools of molecular biology. DNA chips are actually DNA probe arrays. DNA probes (oligonucleotides or long DNA fragments) can be assembled on glass surface, microtiter, plates, plastic sheets, thin polymer gels and semiconductor devices [1].

Using hybridization of DNA samples such as oligonucleotides, cDNA or other cloned genomic DNA fragments to DNA probe arrays, a lot of genetic analytical work will be available, such as analysis of gene expression patterns by monitoring gene expression[2,3], detection of gene and gene mutations[4–6], DNA sequencing[7] and genome physical mapping[8]. Compared with other methods, gene chip has lots of advantages in genetic analysis, such as parallel data processing, miniaturization and automatization. Thus DNA chip or gene chip is playing an increaseingly important role in life science and medical diagnostics. It will be a powerful tool in genome or postgenome research and in many other areas such as drug design, identification of legal medical residue, monitoring of food, health and environment, and military.

DNA probes can also be immobilized on one end of optical fiber, which was studied systematically by Walt[9–11]. Fiber-optic DNA biosensor can be made by this method, and fiber-optic

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DNA biosensor array can be made by assembling these biosensors, each with a different immobilized probe on its tip, and the array can be used to detect gene and gene mutations[1,12]. The fiber-optic DNA biosensor presented here was prepared with a simple method, which was used to make DNA array on slides by DeResi et al.[13]. After the treatment of optical fibers with poly-l-lysine, synthetic oligonucleotide probes can be adsorbed on one end of the fiber, and following the treatment with cold fixative (methanol : acetic acid, 3 : 1), probe can be immobilized on the fiber. The fiber-optic DNA biosensors, which carried different oligo DNA probes, could be assembled in a bundle to form a fiber-optic DNA biosensor array. Fluorescence-labeled target DNA complementary to one or more probes was used to demonstrate the capability of the array.

1 Materials and methods

1.1 Peeling optical fibers

Optical fibers were soaked in sulfuric acid (H_2SO_4) for 30 min to 1 h, then they were taken out carefully and washed with water and dried in air. The skin of the fibers was wiped away with tissue, which had been soaked in absolute ethanol. Then the fibers were cut to 2.5 cm in length and were soaked in chromic acid for 30 min in order to wash up the remaining of the fiber skin. Finally the fibers were rinsed with water and distilled water, and dried in air for the following use.

1.2 Surface treatment of the ends of optical fibers

One end of the optical fiber was soaked in 2 mol/L NaOH / 70% ethanol solution for 2 h, rinsed with distilled water, and dried in air. The treated end of the fibers was soaked in 0.2% poly-l-lysine (Sigma) solution for 1 h, and dried at 40℃ for 30 min[13].

1.3 Adsorption and immobilization of oligo DNA probes on the ends of optical fibers

The gene probes and their sequences used in the experiment are listed in table 1. Perkin Elmer Corporation synthesized the probes of *p53* gene. The probes of *N-ras* gene and *Rb1* gene were synthesized by Shanghai Sangon Bioengineering Company. The ends of the optical fibers which had been treated with poly-l-lysine were soaked in 0.1 mmol/L oligonucleotide probe solution for 15 min. The optical fibers were heated for 5 min at 80℃ in oven, and then immersed in cold fixative (methanol : acetic acid, 3 : 1) for 5 min. The fibers were heated again for 30 min at 80℃. Fixation and heating cycle were repeated 3 times, 5 min each [14]. Then the fibers were washed by soaking them in a 1.5 mL Eppendorf tube according to the following sequence: 0.2% SDS, twice, 5 min each; dH_2O, twice, 5 min each; sodium borohydride solution (1.0 g $NaBH_4$ in 300 mL PBS and 100 mL of 100% ethanol), once, 5 min; 0.2% SDS, twice, 1 min each; dH_2O, twice, 1 min each [15].

Table 1 Oligonucleotide sequence

Name	(Code)	Sequence 5′ – 3′	
p53	probe	GTCTGGCCCC	TCCTCAGC
N-ras	probe	GAAACCTGTT	TGTTGGATAC
Rb1	probe	TGATAATGCT	ATGTCAAGAC
p53	primer 1	ATGGAGGAGC	CGCAGTCAGA
p53	primer 2	GTCTGAGTCA	GGCCCTTCTG

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1.4 Target DNA preparation

The target DNA of *p53* gene was the cDNA abstracted from *p53* cDNA clones, which were cloned in plasmid. The plasmid DNA was abstracted according to the standard method[16]. The target DNA of *N-ras* gene and *Rb1* gene were also cDNA fragment, 0.9 and 3.8 kb respectively, bought from Bejing Tian Xiang Ren Bioengineering Corporation.

1.5 Fluorescent labeling of the target DNA

1.5.1 Labeling of *p53* cDNA. *p53* cDNA was labeled by PCR. The template DNA was the plasmid DNA containing the full length of *p53* cDNA. The primers (table 1) were two oligonucleotides complementary to the coding region of *p53* gene. Perkin Elmer Corporation synthesized the two primers. A PCR kit (Promega) was used to label the *p53* cDNA. The dNTP used for labeling was dCTP-CY5 (0.5 mmol/L) (Pharmacia Corporation). The dNTPs were prepared as follows: take respectively 10 μL dATP (10 mmol/L), 10 μL dTTP (10 mmol/L), 10 μL dGTP (10 mmol/L), 9.5 μL dCTP (10 mmol/L), 10 μL dCTP-CY5 (0.5 mmol/L), 0.5 μL of ddH_2O, and add them to an Eppendorf tube (0.5 mL), mix them well. The ratio of dCTP to dCTP-CY5 in the dNTPs was 19: 1. Add 2 μL plasmid DNA (10 ng/μL), 5 μL PCR buffer (10×), 5 μL $MgCl_2$ (25 mmol/L), 5 μL dNTPs, 0.5 μL primer 1 (100 pmol/μL), 31.75 μL ddH_2O, 0.5 μL primer 2 (100 pmol/μL), 0.25 μL Taq polymerase (4 U/μL), 100 μL paraffin oil to an Eppendorf tube (0.5 mL) for PCR. PCR was carried out according to the following procedure: 94℃, 45 s; 55℃, 120 s; 72℃, 120 s; 25 cycles. The product of PCR was purified according to some normal method for the following steps. The length of cDNA of *p53* gene labeled by PCR was 1179 bp.

1.5.2 Labeling of *N-ras* and *Rb1* cDNA. 1 μg of *N-ras* cDNA (0.9 kb) and 1 μg of *Rb1* cDNA (3.8 kb) were labeled respectively by a High Prime Kit (Boehringer Mannheim). Labeling reaction and the purification of labeled product were carried out according to the protocol of the kit. The fluorescent dye used in the above two labeling reactions was also CY5, conjugated to dCTP (dCTP-CY5), which was ordered from Pharmacia. The ratio of dCTP to dCTP- CY5 in labeling reaction was 19: 1. The length of DNA after labeling by high primer reaction was about 200 — 1000 bp (according to the protocol of the kit). So the length of labeled cDNA of *N-ras* was about 200—900 bp, and the length of labeled cDNA of *Rb1* is about 200—1000 bp.

1.6 Preparation of hybridization solution

1.6.1 Hybridization solution of *p53* gene. The purified DNA labeled with CY5 was dissolved in 50 μL TE buffer and mixed well. Then 2 μl of this solution was taken and added to a 0.5 mL Eppendorf tube. 18 μL hybridization buffer (Boehringer Mannheim)was added to the tube again and mixed well for the following hybridization reaction. The final concentration of the target DNA of *p53* was about at the level of 10 μmol/L by theoretical computing (supposing DNA was amplified 10^6 times by PCR).

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1.6.2 Hybridization solution of *N-ras* gene. After 1 μg cDNA of *N-ras* (0.9 kb, about 1.70 pmol) was labeled, the yield of labeled product was about 1700 μg (see the protocol of the kit), 2.89 pmol. The labeled product was dissolved in 20 μL TE buffer after being purified. 2 μL of the above solution was taken and mixed with 18 μL hybridization buffer. So the final concentration of the target DNA of *N-ras* was 14.5 nmol/L.

1.6.3 Hybridization solution of *Rb1* gene. After 1 μg cDNA of *Rb1* (3.8 kb, about 0.40 pmol) was labeled, the yield of labeled product was about 1700 μg (see the protocol of the kit), about 0.68 pmol. The labeled product was dissolved in 20 μL TE buffer after being purified. 2 μL of the above solution was taken and mixed with 18 μL hybridization buffer. So the final concentration of the target DNA of *Rb1* was 3.2 nmol/L.

1.6.4 Hybridization solution of multi-gene. Take respectively 2 μL labeled DNA solution (dissolved in TE buffer) of *p53*, *N-ras* and *Rb1* and mix them well with 14 μL hybridization buffer. This solution would be regarded as the hybridization solution of multi-gene.

1.7 Preparation of fiber-optic DNA biosensor array

The fiber-optic DNA biosensors which carried different oligo DNA probes could be assembled in a bundle to form a fiber-optic DNA biosensor array, and this DNA array could be used for hybridization to target DNA solutions.

1.8 Application of the fiber-optic DNA biosensor array

The above four hybridization solutions were denatured for 5—10 min at 95—97℃, and quickly set on ice. The fiber-optic DNA biosensor arrays were soaked in the above denatured target DNA solution, hybridized for one night at 62℃[13](The time of hybridization would be adjusted along with the concentration of the target DNA solution. It could be 2 h or 16 h long). After the hybridization reaction, the DNA array was washed by the following procedure[13], and then a good signal-to-noise ratio could be achieved: 2×SSC / 0.2% SDS, RT, twice, 15 min each; 1×SSC/0.1% SDS, 54℃, twice, 15 min each; 0.05×SSC, 54℃, twice, 15 min each. The array was dried in air after the above washing.

1.9 Detection of the hybridization signal on the array

The fiber-optic DNA biosensor array was excited at 633 nm with a He-Ne laser and imaged at 685 nm with a CCD camera. The result of hybridization was analyzed according to the image of the array.

2 Results and discussion

The results are shown in fig. 1. Fig. 1 (a) is the image of the array with white light transmitted through the untreated end of the array. Fig. 1 (b) is the background fluorescence image at 685

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nm taken with 633 nm excitation, with no fluorescence signal observed. The numbers show the order of the fibers in the array. They were respectively (i) naked optical fiber (control); (ii) optical fiber treated only with poly-l-lysine (control); (iii) optical fibers immobilizing *p53* probe complementary to noncoding region of *p53* gene (control); (iv) optical fiber immobilizing *p53* probe complementary to coding region of *p53* gene; (v) optical fiber immobilizing *N-ras* gene probe and (vi) optical fiber immobilizing *Rb1* gene probe.

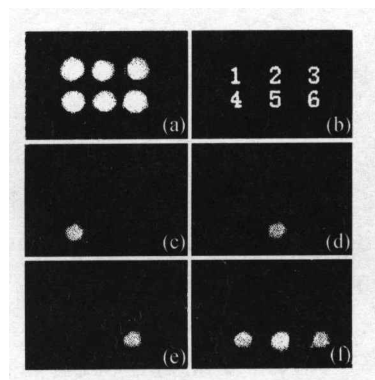

Fig. 1. Image of a fiber-optic biosensor array obtained by a CCD camera. (a) Image with white light transmitted through the untreated end of the array; (b) background fluorescence image at 685 nm taken with 633 nm excitation, no fluorescence signal observed. The numbers show the order of the fibers in the array; (c)—(e) fluorescent image of the fiber-optic biosensor array acquired after hybridization with *p53* target (c), *N-ras* target (d), *Rb*1 target (e); (f) a mixture of *p53, N-ras,* and *Rb1* targets.

The results of fig.1(c)—(f) show that the naked fiber, the fiber treated only with poly-l-lysine and the fiber immobilizing the oligo probes non-complementary to the target DNA sequence have no fluorescent signal after hybridization to any target DNA. Contrast to this result, all of the fibers immobilizing the oligo probes complementary to the target DNA sequence have fluorescent signal after hybridization to the target DNA. The results demonstrate that the fluorescent signals are produced from the hybridization of the oligo probes immobilized on the end of the fiber to the specific target DNA, and so the optical fiber immobilizing specific oligo probes can be used as a fiber-optic DNA biosensor for gene detection. It can detect specifically the unique gene in biological samples. The result of gene detection by this method is accurate and reliable. These results also demonstrate that the method of preparing fiber-optic DNA biosensor is right and viable. Many of these fiber-optic DNA biosensors can be used to form fiber-optic DNA biosensor array, and this DNA array can be applied to detect a large number of genes in the samples simultaneously.

The concentration of the oligo probe used for the attachment on the optical fiber was 0.1 mmol/L. The optical fiber can attach and immobilize enough oligo probes by the method described above and make the fiber-optic DNA biosensor have a high sensitivity of detection.

The concentrations of the three DNA targets for hybridization to the array were respectively a few μmol/L (*p53*), 14.5 nmol/L (*N-ras*) and 3.2 nmol/L (*Rb1*), demonstrating that the sensitivity of this fiber-optic DNA biosensor array for gene detection is at least a few nmol/L, which is higher than that of Walt (10 nmol/L). At this level of concentration of DNA target, a good result can be achieved with 3—5 h of hybridization, so that the overnight hybridization was not needed. But if the hybridization time is less than 2 h, a good result is not often achieved (data not shown). This is different from the result of Walt and his colleagues [1]. In their experiment a good hybridization signal could be achieved when the fiber-optic DNA biosensor array hybridized to

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10 nmol/L target DNA solution only for 10 min[1].

As an effective tool for genomic analysis, the advantage of DNA array lies in its abilities to detect a large number of genes simultaneously. By hybridizing DNA array to mRNA or cDNA from different tissues or cells, the expression patterns of thousands of genes in different tissues or cells at different stages of development can be monitored and analyzed. By analyzing the results of hybridization, we can confirm in which tissues or cells one gene is expressed and which genes are expressed in one tissue or cell, and confirm the difference of time and space of expression of different genes. From these analyses we can find effectively many genes which are only expressed in special time, space or special cell, find useful clues of functions of genes, and even directly confirm the function of gene. Moreover, we can confirm the interaction and coadjustment among genes. So DNA array will be a powerful tool in post-genome research (functional genome research). It will be widely used to find new genes, recognize gene and confirm gene's function. Now we are arranging to use the fiber-optic DNA biosensor array to monitor gene expression in apoptosis of human cancer cell for studying the molecular mechanics and control of cell apoptosis.

In order to not only detect the target DNA but also confirm their exact copies, a quantitative research is urgently needed. So we are trying to improve the sensitivity of detection so that the fiber-optic DNA biosensor array can be used in detection of point mutation.

Despite that our experiment is very primary, the achieved results have shown that the set of protocol we have developed to prepare fiber-optic DNA biosensor and its array is available. On the basis of the above work, we are developing a low-density (about 100 gene probes) linear fiber-optic DNA biosensor array and the relative linear scanning detection system by laser. This linear array maybe plays an important role in functional research and gene diagnosis.

Compared with the other type of DNA microarray, one disadvantage of fiber-optic DNA biosensor array is that it seems not suitable for preparation of high-density gene chips. When the number of DNA probes is above 10 000, the preparation of the fiber-optic DNA biosensor array will be more difficult. But the fiber-optic DNA biosensor array has its advantage on the detection system of the DNA microarray, especially the middle or low-density gene chips. There are many types of methods which can be used as the detection systems of the fiber-optic DNA biosensor array, such as imaging system, scanning system. Since it does not need confocal microscope for detection, the cost of the equipment for detection can be reduced remarkably, and these are favor to the popularization and application of the gene chips in more and more areas.

The method for preparation of optic-fiber DNA biosensor and its array described in this article is very simple. It is suitable for the automatic massive preparation of the biosensors, and so it is more favorable to the commercial development and production of the DNA microarrays.

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