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The portable fluorescence detection system matched with PDMS microfluidic biochip for DNA hybridization detection

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ABSTRACT

The self-made fluorescence detection system was integrated to detect the biological signal of DNA sample. And the portable fluorescence detection system with four channels microfluidic biochip was finished for DNA hybridization.

In the biochip preparation, the PDMS cast-forming process was used to fabricate microfluidic biochip with width 150 μm and height 200 μm. In the fluorescence detection system, four sets of independent 532 nm green lasers were adopted to illuminate the four detection areas of the microfluidic biochip for fluorescence sample excitation, then the voltage of excitation fluorescence signal was obtained. Then the PSOC embedded system was utilized synchronously to display the four voltage values on LCD, in the meantime, the voltage value was transferred to the computer for recording.

From the experimental result of DNA hybridization, it can be seen that portable fluorescence detection system can be utilized to measure four fluorescence signals of DNA samples at the same time. Moreover, due to the hybridization specificity of fluorescence probe ET996-Hex, the DNA hybridization of Edwardsiella tarda BCRC 16711 and Edwardsiella tarda BCRC 16702 can be judged successfully for fluorescence probe ET996-Hex. The portable fluorescence detection system had the capability to distinguish disease source.

1. Introduction

Since Micro-Electro-Mechanical-System (MEMS) technology has very fast development, it is now seen as one of the research fields with the greatest development potential. MEMS system has many advantages, for example, small volume, suitability of mass production and high added value. Meanwhile, its resolution and sensitivity are all higher than that of traditional electromechanical system, hence, MEMS system has been applied as interdisciplinary integrated technology. MEMS system technology has been applied in bio-reaction, and the current biosensor is mainly based on the concept of miniaturization, multi-function and parallel-processing chip; biochip has become one of the development keys in the biomedical related researches. Biochip uses MEMS, semiconductor, biotechnology and other delicate processing technologies; meanwhile, it is accompanied with the application of the principles of gene information, analytical chemistry and molecular biology for the design. It is a device made up of silicon microfluidic chip [1–4], glass or polymer material. It can be applied in biological reaction or analysis, which is one very important technology in bio technology. It is a development concept toward miniaturized lab. The preparation technology is complicated, but it is pretty convenient to the user, that is, the user only needs to inject the sample to be tested directly into the chip to perform automatically the complicated experimental process flow, meanwhile, several samples can be processed on the same chip at the same time, and several reactions can be carried out at the same time too, that is, the test result can be obtained quickly.

Biochip has wide application scope, and its reaction target can be cell tissue, gene or protein, etc. It has many applications in biomedical research and new medicine development. It can enhance medical diagnostic capability, it can also reinforce the detection of environmental monitoring; meanwhile, it can also make the detection of forensic science and farming foods more efficient. Its main features are: higher analytical speed, less use of sample and reagent, high sensitivity, high specificity, the acquisition of highly precise and reliable experimental data, hence, many countries around the world invests aggressively in related bio technology industries, and biochip industry is also seen as the star industry of the future.

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The biochip can be divided into two parts. The first part is the micro total analysis system (μ-TAS); the second part is the fluorescence detection system. In 1990, professor Manz had proposed the concept of μ-TAS to use MEMS technology to integrate the complicated detection and operation process of a lab into a chip of only a few centimeters, and this is the basic structure of lab-on-a-chip [5]. In 1997, Man P.F. et al., in the application of microfluidic system, used the concept of Lab-on-a-Chip and used the MEMS technology to miniaturize the analytical instrument and to integrate it in a small area biomedical chip, in this way, a biomedical chip of different functions such as mixing, deoxyribonucleic acid (DNA) replication, fluorescence detection, etc. is made.

In biomedical related research field, it is hoped that MEMS device can be made with wider applications hence, an innovative process that is different than semiconductor silicon-based process is needed so as to achieve the characteristic of convenience and speed, hence, soft lithography technology. In 1974, Bell lab, the first place to develop elastomeric micromolding technology, started to use soft material as the master mold of lithography process. In 1998, the research team of Whitesides announced formally soft lithography technology. Soft lithography technology is a process technology suitable for microfluidic device, and it is mainly developed from two methods of rapid prototyping and replica molding [6], in 2001, professor Whitesides even announced related research to apply soft lithography technology in biological and biochemical field. In 2003, Gwo-Bin Lee et al. proposed the use of He-Ne laser as excited optical source, then through the use of optical fiber coupling to optical source, the light was guided to avalanche photodiode (APD) module to be used as cell counter; meanwhile, through the measurement of the output voltage change of APD module caused by the interruption of excited optical source by cell, the number of cell was measured [7]. In 2002, Jan Kruger et al. used short wavelength light-emitting diode (LED) accompanied with red semiconductor laser as excitation optical source, then the APD chip was used to receive the light, and the fluidic chip was prepared separately. It had the effect of low cost and miniaturization at the same time, meanwhile, light of different wavelength can also be taken as excitation optical source, hence, different fluorescence dye can be used to label and to measure different biological samples [8]. Currently, many biological tests and analyses are carried out in fluorescence detection way, in typical fluorescence detection instrument there is an excitation optical source and a signal receiving part. The fluorescence detection system will be accompanied for experiment so as to complete the processing, reaction or analysis and detection of all kinds of samples [9–18].

Biochip originated in 1980s, the earliest technology can be dated back to the finding by EdSouthern that labeled nucleic acid can be paired and hybridized by another solidified nucleic acid molecule. BainsW et al., in 1990s, fixed short DNA fragment onto a support, then through hybridization way, sequence test was performed. In 1992, the research team of Harrison and Manz, at Ciba Geigy Corporation had developed the first μTAS. The association of MEMS system with μ-TAS systems for all kinds of chemical analyses has many advantages, for example, fewer sample demand, shorter detection time, easier system integration, and the enhancement of detection system capability [19]. The precursor Affymax of biochip leader of US Affymetrix, further applied the photo-chemical technology of semiconductor technology to prepare high density nucleic acid probe array. The development of biochip, according to function, can be divided into two main streams, namely, microarray and processing type biochip (Lab-on-a-chip) that emphasizes on functional integration:

Microarray: For example, protein chip and gene chip, etc. Microarray chip is to grow high density biological probe on very tiny area of substrate. It is used as tool for massive sorting and parallel analysis. Meanwhile, it is aligned on special slot or film material of biochip through automated mechanical way, for example, inkjet printing [20–22] or spotting [23–25], etc. Microarray has characteristics such as convenience, speed, and time-saving, which is suitable for researches such as massive gene expression, sorting and comparison, etc. It can be used in the gene detection of the origin of disease, gene mutation analysis, gene expression comparison, gene sequence analysis, and new medicine development, etc. Microprocessor chip is also called processing microchip. For example, Lab-on-a-chip and Microfluidic chip, etc. Microprocessor chip can be used to process biological sample and to carry out biological reaction or used as tool for analyzing biological body. The pre-treatment chip for sample can be used to treat blood, tissue and plant sample to reduce the contamination and risk during human operation; reaction type chip can be used to carry out micro organic chemical reaction, micro organic chemistry reaction, enzyme reaction, or biological reaction; in addition, analytical chip can be used to perform capillary electrophoretic or high speed sorting reaction. The greatest advantage of microprocessor chip is to carry out the pre-treatment, reaction or analytical procedure of the sample in a tiny space so as to achieve the function of integration.

The main objective of this research is to use MEMS process technology to prepare microfluidic biochip; the process includes mask design and drawing as well as optical development technology, etc.; using that process, we can duplicate master mold with stereo structure to a polymer material Polydimethylsiloxane (PDMS) so as to prepare biochip with stereo microfluidic structure. Since this type of polymer material has the characteristic of easy to form and low cost, usually, it can be duplicated in massive way through the use of glass, silicon wafer and polymer as master mold.

Usually, the use of biomedical analytical and detection system will take lots of time, and it needs the operation by professional personnel at specific lab. Moreover, the sample amount needs to be taken is a lot, the detection step is very tedious and complicated, no matter in experiment or in detection aspect, it will take lots of time cost and human cost, hence, it is hoped that through MEMS system technology, the utilization difficulty can be improved, and the detection convenience and accuracy can be increased. If MEMS technology can be successfully used to miniaturize detection equipment in the lab and to further integrate it into a chip of only several centimeters size so that the chip can carry out jobs such as biochemical reaction, separation and detection, etc. at the same time, then it will possess the advantages such as: small volume, fast detection speed, usage of only small amount of specimen and sample, easy automation, etc. That is, the detection job can be made simpler, more accurate, in the mean time, the detection time can be shortened, and the biological detection efficiency can be enhanced too. The current optoelectronic detection system is usually of bulky volume and high cost, the complicated equipment not only will retard the development of portable detection system but also will greatly increase the detection cost. Hence, many researches have proposed the feasibility to miniaturize biomedical analytical and detection system, that is, through the miniaturization of detection system, the detection convenience is increased, meanwhile, accompanied with the disposable microfluidic chip it is made more advantageous in lab cost and utilization convenience.

In this research, optoelectronic detection system will be self-prepared, that is, fluorescence detection system such as green laser (532 nm) and optical sensor, etc. will be set up, and it will be provided with test sample to conduct fluorescence detection test. Meanwhile, through interdisciplinary integration of portable fluorescence detection system, it can be used as fluorescence detection of biochip, that is, it can replace traditional complicated labor job and high cost large equipment, hence, the objectives such as easy detection, low sample amount, low cost, easy utilization as well as the promotion of the miniaturization of biomedical analysis and detection can be achieved.