INTRODUCTION TO DNA MICROARRAYS AND THE PILOT STUDY OF AN ACTIVE DNA MICROARRAY FABRICATION IN THAILAND

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ABSTRACT

Biomedical research evolves and advances through the development of new technologies. Using traditional methods, researchers are able to survey a relatively small number of genes at a time. DNA microarray technology allows scientists to analyze a very large numbers of nucleic acid fragments in a single experiment quickly and efficiently. This paper introduces the DNA microarray technology, and current available fabrication techniques. The available fabrication techniques include microspotting, photolithography and ink-jetting. Our ongoing research on the pilot DNA microarray fabrication is discussed. A design and fabrication process using microfabrication technique of an active DNA microarray, a part of our ongoing research, is described.

Index: DNA microarrays, active DNA microarrays, microelectronics, microfabrication, gene expression, hybridization

1. INTRODUCTION

More than four hundred diseases are currently diagnosable by molecular analysis of nucleic acids. A man has approximately 100,000 genes that could be potentially tested for defects or diseases. In the past, gene detection using DNA hybridization can be done only a few genes at once. In this technique, the DNA probe is labeled single-stranded DNA to provide detectable signals, however this traditional radioisotope methods are not applicable to regular environments [1, 2]. The non-radioactive labeling techniques [3, 4], such as the polymerase chain reaction (PCR), have been developed to replace the traditional methods. However, the cost of these methods is too high to be used widely in general. Recently, there has been much interest in the microfabrication of microelectromechanical devices for genetic assays. These devices are excellent candidates because of their performances and costs. The genetic assays can be determined and improved in the micro-scale, and these micro-parts can be used for many different assays by only changing the nature of its reagents while their constructions are still the same. The development of DNA microarray technology allows scientists to examine thousands of genes at the same time with great efficiency.

Section 2 discusses the DNA microarray technology, and Section 3 reviews available microarray fabrication techniques, included microspotting, photolithography and ink-jetting techniques. Sections 4 and 5 discuss our ongoing research on an active DNA microarray fabrication using microfabrication techniques. These include design and fabrication processes. Finally, a discussion is presented in Section 6.

2. DNA MICROARRAYS TECHNOLOGY

In literatures, DNA Microarrays are called in various names, such as, DNA/RNA Chip, BioChip or GeneChip. DNA microarrays consist of a collection of DNA sequences or probes deposited in an ordering arrangement on a solid surface, such as a glass slide, silicon wafer or membrane. Each DNA probe is complementary to a DNA sequence within one or more genes. The DNA used to create a microarray is often from a group of related genes such as those expressed in a particular tissue, during a certain developmental stage, in a certain pathway, or after treatment with drugs or other agents [5]. Expression of a group of genes is quantified by measuring the hybridization of fluorescently labeled RNA or DNA to the microarray-linked DNA sequences. By profiling gene expression, transcriptional changes can be monitored through organ and tissue development, microbiological infection, and tumor formation. All of the genes in genome can be arrayed in an area no larger than a standard microscopic slide. The DNA microarray is used to determine gene activity within a cell by indicating which genes are being expressed and to what degree. There are different methods for depositing the nucleic acid sequences onto microarray support (see [6].)

Three major steps involve in a typical experiment in DNA microarray technology; 1) create array and preparation of microarray, 2) preparation of fluorescently labeled probes and hybridization, and 3) washing, scanning image and data analysis.

Microarrays are available in two different format; 1) Oligonucleotide arrays and 2) cDNA arrays, which use the different methods above for depositing the nucleic acid sequences onto microarray support. In microarray analysis, the different gene expression is analysed by co-hybridising fluorescently labeled cDNA probes prepared from two different RNA sources. The

labeling procedure involves the conversion of mRNA to cDNA and labeling the cDNA with fluorescent dyes. The most frequently used fluorescent dyes are Cy3 (green) for control samples and Cy5 (red) for test samples. The product of the labeling reaction can be analysed spectrophotometrically by measures the nucleotide/dye ratio.

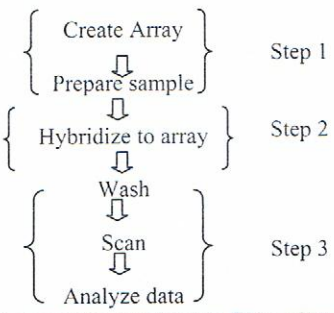


Fig 1. Three primary steps involved in DNA microarray technology

3. PRESENT FABRICATION TECHNIQUES

Microfabrication for DNA microarrays fabrication is a process used to construct physical objects with dimensions in the micrometer to millimeter range [7]. The three primary technologies currently used in microarray fabrications.

3.1 The microspotting technology

Patrick Brown of Stanford University invents the microspotting technique, which relies on direct surface contact for microarray fabrication. In this method, long DNA molecules (cDNA) are deposited by high-speed robots on a solid surface. Solid and hollow (split-open) pen designs (Fig 2) are used to transfer target nucleic acid onto the supporting surface. The pen is dipped into the target solution and a small volume of the solution adheres to the pen. When the pen comes into contact with the supporting surface, it transfers a fraction of nucleic acid solution onto solid surfaces.

3.2 Photolithography technique

The photolithography approach uses the same technology for making semi-conductor chip. Most DNA microarrays are fabricated onto glass or plastic wafers, or are placed in tiny glass tubes and reservoirs [8]. This method was firstly developed at the Affymetrix Inc., called "DNA chips," which also known under the *GeneChip®* trademark. Affymetrix uses several photomasks and lighting processes to expose reaction positions selectively on silicon plates, then attaching the oligonucleotide onto the plates. An oligonucleotide (or oligo) is a short fragment of a single-stranded DNA that is approximately 20-25 nucleotide bases long. Oligo synthesis begins by attaching chemically modified linker groups, which contain photochemically removable protective groups, onto the glass surface [9]. A supporting surface is covered with a photoactive mask, and when lights are selectively rayed through masks reactant groups are exposed and can react with following units. In each step (Fig 3), the unprotected areas are first activated with light which removes the light sensitive protective groups. Exposure of the activated area results in chemical attachment of the nucleoside base to the activated positions. A new mask pattern is applied. This process is then repeated and a new nucleotide has been added to the oligomers.

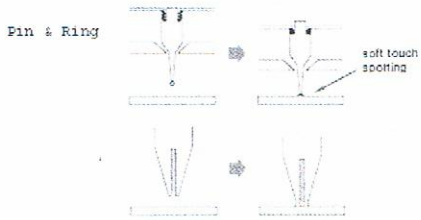


Fig 2. Solid and hollow pen designs for microspotting (Courtesy http://www.bohan.co.kr/html/DNAChip/DNA Chip-1.htm)

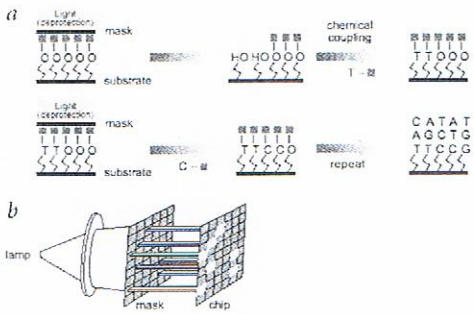


Fig 3. Microarray manufacturing using photolithography (Courtesy Lishutz et al, 1999)

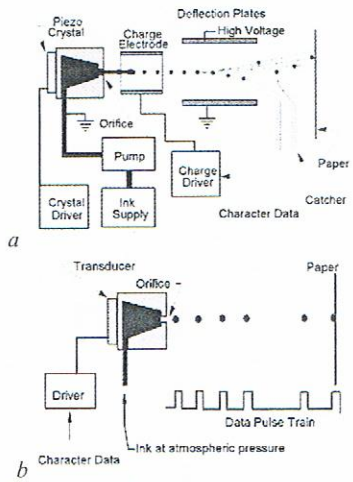


Fig 4. a) Schematic of continuous mode ink-jet printing system. b) Schematic of a drop-on-demand ink-jet printing system.

3.3 Ink-jetting technology

The ink-jetting technology is a non-contact, data-driven deposition method which combining DNA synthesis chemistry with commercial ink-jet technology. Using this

technology the spheres of fluid can be dispensed with diameters of 15 to 100 μm at rate of 0-25,000 per second from a single drop-on-demand and up to 1 mHz for continuous droplets print head [10]. Most of these methods divide into two general categories; 1) continuous mode and 2) demand mode.

Continuous mode ink-jet printing systems (Fig 4a) produce droplets size of approximately twice the orifice diameter. Droplet generation rates for commercially available continuous mode ink-jet systems are usually in the 80-100 kHz range (1 MHz also available.) Droplet sizes can be as small as 25μm in a continuous system, but the size of 100μm is typical. MicroFab has built systems that produce droplets as large as 1 mm (~0.5μl).

In demand mode ink-jet (Fig 4b), the fluid is maintained at ambient pressure. A transducer is used to create a drop only when needed. Volumetric changing in the fluid is induced by the application of a voltage pulse to a piezoelectric material that is coupled, directly or indirectly, to the fluid. This volumetric change creates pressure waves. The pressure waves travel to the orifice, are converted to fluid velocity, which results in a drop being ejected from the orifice [11–13]. In most commercially available ink-jet printing systems today, a thin film resistor is substituted for the piezoelectric transducer. When a high current is passed through this resistor, the ink in contact with it is vaporized, forming a vapor bubble over the resistor [14]. This vapor bubble creates a volume displacement in the fluid and serves the same functional purpose as the piezoelectric transducer. Since the voltage or the current is applied only when a drop is desired, these types of systems are referred to as drop-on-demand, or "demand mode." Demand mode ink-jet printing systems produce droplets that are approximately equal to the orifice diameter of the droplet generator [15].

4. DESIGN OF AN ACTIVE DNA MICROARRAY

Our ongoing research is to firstly fabricate an active microarray in Thailand. Active microelectronic arrays for DNA hybridization analysis (commonly known as 'active DNA microarray') take advantage of advanced microfabrication processes developed by semi-conductor industry. Active DNA microarrays have major advantages over traditional passive DNA microarray, which are: rapidly transport and address DNA probes to positions on the array surface, accelerate the basic hybridization process, and rapidly discriminate single base mismatches in the target DNA probes [16]. This section describes our design of an active DNA microarray chip. Figs 5 and 6 illustrate photo masks 1 and 2, respectively for our designed chip fabrication. The next section describes our plan of the fabrication process in fabricating our prototype.

5. FABRICATION PROCESS

The fabrication process is shown in Figures and is described below:

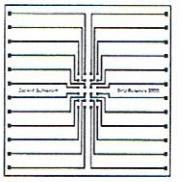


Fig 5. Mask-1 containing the image of the desired pattern of electrodes (1st Photolithography).

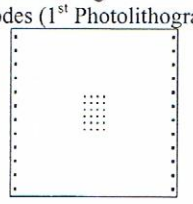
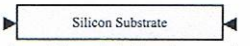
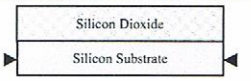


Fig 6. Mask-2 containing the image of the desired pattern of test sites and bonding pads (2nd Photolithography).

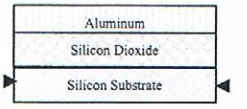
(1) The starting material is a silicon wafer, with a thickness of 350-400 microns. Silicon wafer is cleaned with RCA-1 solution.



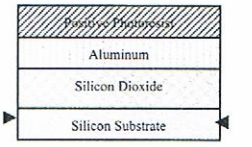
(2) A thermal oxide of 0.5 microns is grown in a high temperature furnace at 1100 °C for 60 minutes.



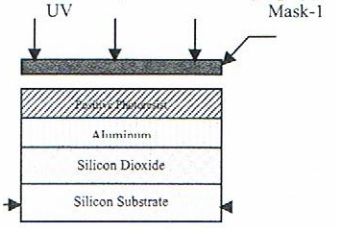
(3) A layer of Aluminum about 3 microns thick is deposited on the substrate by filament evaporation.



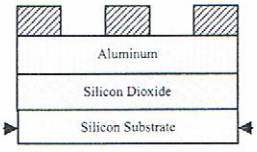
(4) The aluminum surface is coated with a 0.5-microns-thick layer of positive photoresist by photoresist spinner.



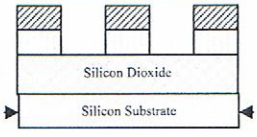
(5) The photoresist is exposed to ultraviolet light through a mask containing the image of the desired pattern of electrodes (1st Photolithography).



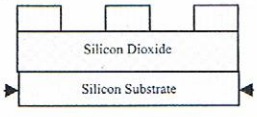
(6) After developing, a protective layer of photoresist with the electrode pattern remained on the metal.



(7) The aluminum is then removed from the unprotected areas by wet etching (using PAN etch).

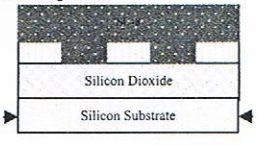


(8) The remaining photoresist is washed away with isopropanol and acetone.

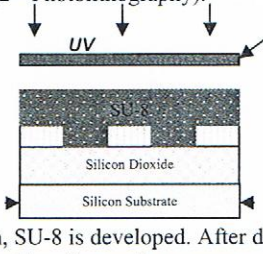


(9) Then, the wafer is passed an aluminum-annealing process.

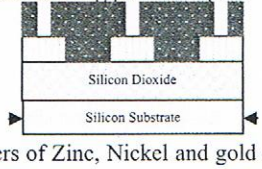
(10) SU-8 negative photoresist is deposited by photoresist spinner.



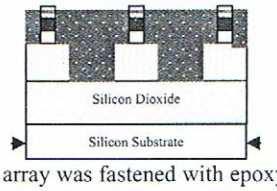
(11) The SU-8 is exposed with a mask containing the image of the desired pattern of test sites and bonding pads (2nd Photolithography).



(12) Then, SU-8 is developed. After developing, the test sites and bonding pads are open.



(13) Layers of Zinc, Nickel and gold are deposited with the following techniques; Techni En Zincate, Alkaline Electroless Nickel Strike-Techni En 9185, and Oromerse SO, respectively (see [9] for details).



(14) The array was fastened with epoxy glue to a circuit board. Aluminum wires are ultrasonically welded to the bonding pads of each microelectrode.

(15) Epoxy glue is used to form a culture dish with the bottom being the electrode array. The wires are insulated and mechanically protected with a layer of epoxy glue.

LABELS:

Gold, Nickel, Zinc, SU-8, Aluminum, SiO_2, Positive Photoresist, Silicon Substrate

6. DISCUSSION

The demand for genetic information is unlimited. Assay cost and time can be reduced by several orders of magnitude if the size of sample and analysis device are reduced to micro-scale as "DNA chip." Though significant progress has been made, the study of DNA microarray is still at early stage. The catalytic, electrical, magnetic, and electrochemical properties of such structures are still ongoing investigations. It is anticipated that new phenomena and useful structures will continue to emerge over the next few years.

This paper introduced DNA microarray technology, and reviewed current microarray fabrication techniques. Our design and plan of fabrication process were described. These were parts of our ongoing research on a pilot study on DNA microarray fabrication in Thailand.

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