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DNA Microarrary: An overview

Dr. S Amutha [1*], Dr. R Selvakumar [2], Dr. Sai Lakshmi LJ [3]
[1] Senior lecturer, Department of Oral Pathology and Microbiology, Dr MGR Educational and Research institute, Thai Moogambigai Dental College and Hospital, Chennai, Tamil Nadu, India
[2] Reader, Department of Oral and Maxillofacial Surgery, Asan Memorial Dental College and Hospital, Chengalpattu, Tamil Nadu, India
[3] Reader, Department of Oral Pathology and Microbiology, Dr MGR Educational and Research institute Thai Moogambigai Dental College and Hospital, Chennai, Tamil Nadu, India

* Corresponding Author: **Dr. S Amutha**

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Abstract
Microarrays are a technology in which thousands of nucleic acids are bound to a surface and are used to measure the relative concentration of nucleic acid sequences in a mixture via hybridization and subsequent detection of the hybridization events. In the past several years, a new technology known as microarray has attracted tremendous interest among biologists and biomedical researchers. This paper briefly discusses the overview of DNA microarrays, history, its principle, types, limitations and its future.

Keywords: DNA microarrays, Spotted arrays, oligonucleotide arrays, Self-assembled arrays

Introduction
An "array" is a systematic arrangement of similar objects, usually in rows and columns. DNA microarray (*DNA chips, Biochips, Gene, Genome chip, Gene array)* technology may be defined as a high-throughput and versatile technology used for parallel gene expression analysis for thousands of genes of known and unknown function or DNA homology analysis for detecting polymorphisms and mutations in both prokaryotic and eukaryotic genomic DNA [1].

History
DNA array was created with the colony hybridization method of Grunstein and Hogness (1975). In 1979, this approach was adapted to create ordered arrays by Gergen *et. al*. who picked colonies into 144 well microplates [2]. In the late 1980's and early 1990's Hans Lehrach's group automated these processes by using robotic systems to rapidly array clones from microtiter plates onto filters. A complete eukaryotic genome (*Saccharomyces cerevisiae*) on a microarray was published in 1997 [3]. Affymax produce the first DNA chips. In the late 90's and 2000's, DNA array technology progressed rapidly as both new methods of production and fluorescent detection [4].

Microarray Principle
mRNA is an intermediary molecule which carries the genetic information from the cell nucleus to the cytoplasm for protein synthesis. Whenever some genes are expressed or are in their active state, many copies of mRNA corresponding to the particular genes are produced by a process called transcription [5]. These mRNAs synthesize the corresponding protein by translation. So, indirectly by assessing the various mRNAs, we can assess the genetic information or the gene expression [5]. This helps in the understanding of various processes behind every altered genetic expression. Thus, mRNA acts as a surrogate marker. Since mRNA is degraded easily, it is necessary to convert it into a more stable cDNA form. Labeling of cDNA is done by fluorochrome dyes Cy3 (green) and Cy5 (red). The principle behind microarrays is those complementary sequences will bind to each other [5].

The unknown DNA molecules are cut into fragments by restriction endonucleases; fluorescent markers are attached to these DNA fragments. These are then allowed to react with probes of the DNA chip [2]. Then the target DNA fragments along with complementary sequences bind to the DNA probes. The remaining DNA fragments are washed away. The target DNA pieces can be identified by their fluorescence emission by passing a laser beam. A computer is used to record the pattern of fluorescence emission and DNA identification [5].

Types [6]

- Spotted arrays (cDNA arrays)
- *in-situ* synthesized arrays (oligonucleotide arrays)
- Self-assembled arrays

Glass cDNA microarrays

Glass DNA microarrays was the first type of DNA microarray technology developed. It was pioneered by Patrick Brown and his colleagues at Stanford University and is produced by using a robotic device, which deposits (spots) a nanolitre of DNA (50-150 µm in diameter) onto a coated microscope glass slide surface in serial order with a distance of approximately 200-250 µm from each other, one spot one gene [7]. These moderate sized glass cDNA microarrays also bear about 10,000 spots or more on an area of 3.6 cm^2. It uses specially manufactured glass slides with desired physico-chemical characteristics e.g. excellent chemical resistance against solvents, good mechanical stability (increased thermal strain point) and low intrinsic fluorescence properties.

The first step of manufacturing a glass cDNA microarray is selecting the material to spot onto the microscope glass surface e.g. the genes from public databases/repositories or institutional sources [7]. This is followed by the preparation and purification of DNA sequences representing the gene of interest. In the preparation process, PCR is used to amplify the DNA from library of interest using a universal primer or gene specific primers and the purity of the DNA fragments representing genes of interest are generally checked by sequencing or using on agarose gel to concomitantly obtain an estimate of the DNA concentration. This is an important step because all the DNA fragments should be of similar concentration/molarity and size, to achieve similar reaction kinetics for all hybridisations. The third step is spotting DNA solution onto chemically modified glass slides usually with poly(L-lysine) or other cross-linking chemical coating materials such as polyethyleneimine polymer p-aminophenyl trimethoxysilane/diazotization chemistry and dendrimeric structure [6]. It is these substrates that are coated on the surface of the glass slide that determines how the DNA solution will be immobilised on the surface e.g. covalent or non-covalent. However, in the course of poly(L-lysine) the negatively charged phosphate groups in the DNA molecule, form an ionic bond with the positively charged amine – derivatised surface. This spotting step is achieved via a contact printing using precisely controlled robotic pins or other equivalent delivering technology such as inkjet printing. The last step of manufacturing glass DNA microarrays is the post-print processing step involving the drying of the DNA on the slide overnight at room temperature and the use of UV cross-linking to prevent subsequent binding of DNA, and to decrease the background signal upon hybridisation of a labelled target [6].

Steps [7]

1. Sele cting the material
   (Libraries of PCR products or long oligonucleotides)
2. PCR is used to amplify the DNA
3. Purity of the DNA fragments representing genes of interest are checked
4. Spotting DNA solution onto chemically modified glass slides usually with poly(L-lysine) or other cross-linking chemical coating materials by contact printing or inkjet printing
5. Post-print processing

Advantages of cDNA Microarrays [6]

- Advantages of Glass cDNA microarrays include their relative affordability with a lower cost.
- Its accessibility requiring no specific equipment for use such that hybridisation does not need specialised equipment, and data capture can be carried out using equipment that is very often already available in the laboratory and flexibility of design as necessitated by the scientific goals of the experiment.
- Glass cDNA microarrays also have increased detection sensitivity due to longer target sequences (2 kbp).

Disadvantages of cDNA microarrays

- Despite their wide spread use, glass cDNA microarray has a few disadvantages such as intensive labour requirement for synthesising, purifying, and storing DNA solutions before microarray fabrication. Further, more printing devices required thus making microarrays more expensive.
- Also during microarray experiments in the laboratory, sequence homologies between clones representing different closely related members of the same gene family may result in a failure to specifically detect individual genes and instead may hybridise to spot(s) designed to detect transcript from a different gene. This phenomenon is known as *cross hybridisation* [8].

Oligonucleotide array

- *In situ* (on chip) oligonucleotide array format is a sophisticated platform of microarray technology which is manufactured by using the technology of *in situ* chemical synthesis - Stephen Fodor *et al*. (1991) [9]

Making of gene chip

- ***Photolithography*** and ***combinatorial chemistry*** is used to manufacture short single strands of DNA onto 5-inch square quartz wafers
- A photolithography mask is used to control light-directed DNA synthesis chemistry such that oligo sequences are built up one nucleotide at a time at defined locations on a solid substrate or glass chip (Figure - 1) [5].

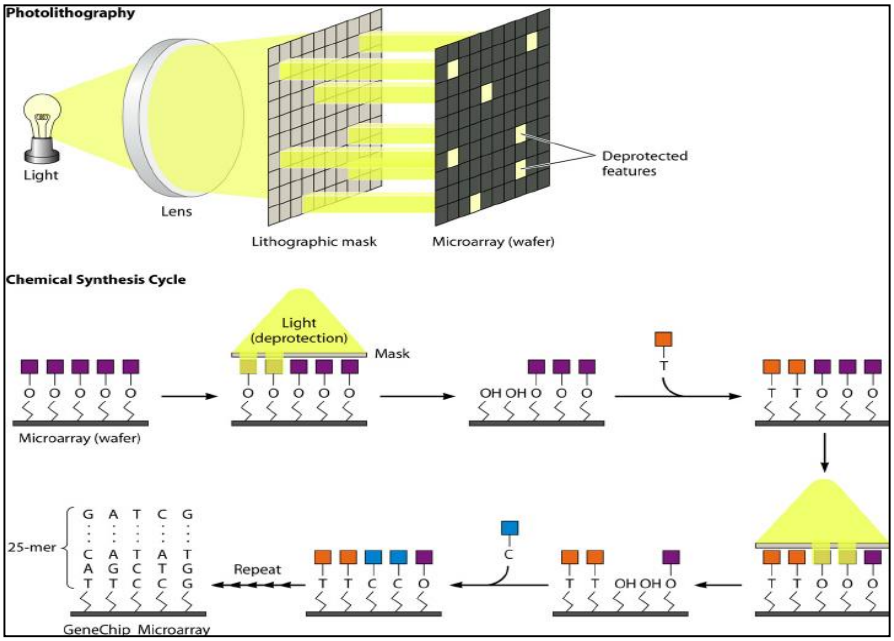

Fig 1: Photolithography [5]

1994, Fodor et.al. Affymetrix, used photolithographic masks in order to direct the light to the array at each step of the synthesis process. In 2002, Nimblegen Systems Inc., used micro-mirrors to direct light instead of using the mask. In 1996, Blanchard et.al. used Inkjet printing technology[8].

Advantages

- Specificity and reproducibility – High
- Easy /quick to generate array. Because, spotting the DNA onto the chip requires only that DNA sequence of interest to be known

Disadvantages

- Prior knowledge of the genome sequence is required
- Need expensive specialised equipment
- Decreased sensitivity/binding compared with glass cDNA microarray - Low sensitivity, compensated for by using multiple probes.

Table 1: Comparison of affymetrix (oligonucleotide) microarrays and cdna microarrays

	Affymetrix microarrays	cDNA microarrays
Manufacture process	Photolithography	Robotic spotting intact clones
Substrate	Glass slide	Glass slide
Probe type	DNA oligonucleotides	cDNA clones
Probe length	25 base pairs	100–1000s base pairs
Label	Single fluorescent dye	Cy3 and Cy5 fluorochromes
Scanner	Laser scanner	Laser scanner
Unit of expression	Average difference	Ratio (Cy5/Cy3)

Self assembeled arrays

An alternative approach to the construction of arrays was created by the group of David Walt at Tufts University (Ferguson *et al*., 2000; Michael *et al*., 1998; Steemers *et al*., 2000; Walt, 2000) and ultimately licensed to Illumina [10]. Their method involved synthesizing DNA on small polystryrene beads and depositing those beads on the end of a fiber optic array in which the ends of the fibers were etched to provide a well that is slightly larger than one bead (figure 2) [10, 11]. Different types of DNA would be synthesized on different beads and applying a mixture of beads to the fiber optic cable would result in a randomly assembled array. In early versions of these arrays, the beads were optically encoded with different fluorophore combinations in order to allow one to determine which oligo was in which position on the array (referred to as "decoding the array"). Later versions of the Illumina arrays used a pitted glass surface to adapt these beads instead of a fiber optic array [10].

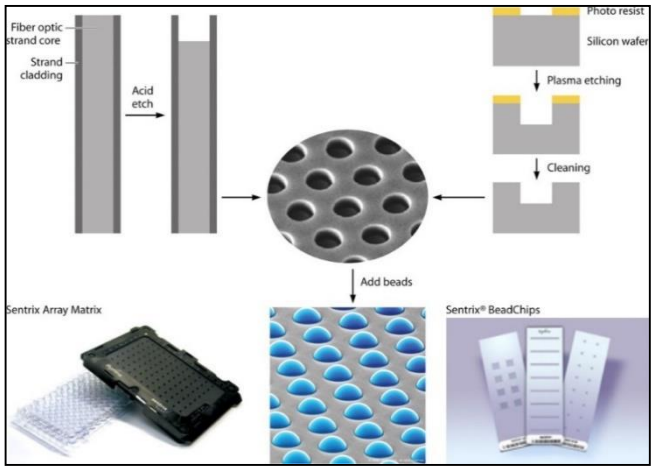

Fig 2: Self Assembeled Arrays [10]

Applications of microarrays

- As a *gene expression* profiling tool [12]
- *Comparative genomics* to analyse genomic alterations such as sequence and single nucleotide polymorphisms (SNP) [13]
- Transcription factor binding analysis

Gene expression analysis

- To measure gene expression levels
- ✓ RNA is extracted from the cells of interest
- ✓ Converted to a labelled cDNA/ cRNA
- ✓ Hybridized to the microarray

- ✓ Increases our basic understanding of the cause and consequences of a disease (pathogenesis)
- ✓ Helps in detection of precancerous lesions
- ✓ To identify appropriate targets for therapeutic intervention

Comparative genomics [14]

- To analyse genomic alterations such as sequence and single nucleotide polymorphisms (SNP).
- To compare between a fully sequenced genome and an unsequenced but related genome.

Transcription factor binding analysis

Microarrays have also been used in combination with chromatin immunoprecipitation [15] to determine the binding sites of transcription factors [16]. In brief, transcription factors (TFs) are cross linked to DNA with formaldehyde and the DNA is fragmented. The TF(s) of interest (with the DNA to which they were boud still attached) are affinity purified using either an antibody to the TF or by tagging the transcription factor with peptide that's amenable to affinity chromatography (for example a FLAG-, HIS-, myc or HA-tag). After purification, the DNA is released from the TF, amplified, labeled and hybridized to the array. This technique is commonly referred to as "ChIP-chip" for Chromatin Immuno-Precipitation on a "chip" or microarray [16].

Limitations of microarray [16]

- *Indirect* measure of relative concentration (DNA/RNA)
- *Difficult to design arrays for complex mammalian genomes*, in which multiple related DNA/RNA sequences will not bind to the same probe on the array
- Only detect sequences that the array was designed to detect

Future of arrays

The advent of next generation sequencing technologies combined with the rapid decrease in the cost of sequencing has now made sequencing cost competitive with microarrays for all assays with the possible exception of genotyping [2]. When the cost is similar, sequencing has many advantages relative to microarrays. Sequencing is a direct measurement of which nucleic acids are present in solution. One need only count the number of a given type of sequences present to determine its abundance [17]. Counting sequences is linear with concentration and the signal to noise one can obtain by sequencing is only limited by the number of reads used for each sample. Sequencing is a relatively unbiased approach to measuring which nucleic acids are present in solution. While sample preparation or different enzymes may bias sequencing counts, unlike DNA arrays, sequencing is not dependent on prior knowledge of which nucleic acids may be present. Sequencing is also able to independently detect closely related gene sequences, novel splice forms or RNA editing that may be missed due to cross hybridization on DNA microarrays [17].

As a result of these advantages and the decreasing cost of sequencing, DNA arrays are being rapidly replaced by sequencing for nearly every assay that has been previously performed on microarrays.

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