REVIEW ARTICLE

Microarray: An Emerging Diagnostic Tool in Dentistry

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ABSTRACT

Aim: Present paper intends to discuss the basic concept for understanding the potential of microarray technology in dentistry.

Summary: Microarray technology, developed in 1990s from southern blotting, while transforming the biological sciences, permitted the global gene expression profiling done in a single experiment. With microarray technological advances, individual experiments create thousands of observations, turning the experiments from a "hypothesis-driven endeavor" to a "hypothesis-generating endeavor", as experiment sheds light across the entire terrain of gene expression, letting relevant genes reveal themselves. Microarrays have made important contributions to both basic and applied research with promise to change future practice of medicine, as well as dentistry.

Keywords: DNA, gene expression, genomic, microarray

INTRODUCTION

Genetic information, present in living organisms in the form of DNA or RNA, identifies an organisms genotype and potential phenotype, and this genomic analysis of multifactorial diseases in human beings may rely on suitable experimental procedures.[1] More recently, the completion of the human genome sequences has opened the door for researchers to extend their studies, seeking genetic factors that influence complex disorders involving multiple genes.[2] As per Oxford Dictionary, microarray is described as a set of DNA segments of known sequence representing the complete set of genes of an organism, arranged in a grid pattern for use in genetic testing.[3] It is a hybridization technique that allows simultaneous analysis of thousands of samples on solid substrates.

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Microarray technology, developed in 1990s from southern blotting, while transforming the biological sciences, permitted the global gene expression profiling done in a single experiment.[4] DNA microarray analysis techniques were first used for the simultaneous measurement of differential gene expression[5] of forty five *Arabidopsis* genes by Schena et al. in 1995.[6]

Microarrays offer an unprecedented view into the biology of DNA, and thus provide a rich way to examine living structures. In the past, researchers had to work hard to obtain little amount of data that could be used to formulate a hypothesis with one observation at a time. However now, with microarray technology, individual experiments create thousands of data points or observations, turning the experiments from a "hypothesis-driven endeavor" to a "hypothesis-generating endeavor", as experiment sheds light across the entire terrain of gene expression, letting relevant genes reveal themselves.[7] This technology potentiates monitoring of the whole genome on a single chip and gives us integrated insight into the processes in the organism. Present paper is thus intended to discuss the basic principle for understanding the potentials of DNA microarray technology in dentistry.

PRINCIPLE OF MICROARRAY

A DNA microarray (synonyms DNA chip or DNA array) can be thought of as a miniaturized form of dot-blot, but in a high-throughput format[8] or miniaturized gene-hybridization or gene-detection assay.[9] DNA microarrays represent DNA fragments attached to the surface in a predefined ordered fashion at high density. The principle of microarray experiments is that the mRNA or total RNA from given cells or tissue is used to produce a labelled sample, which is hybridized in parallel with a large number of DNA sequences immobilized on a solid surface in an ordered array.[6] Global analysis of

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thousands of gene expressions may be performed with the accessibility of genome information and development of microarray method in a single assay.[10,11] Each microarray experiment typically follows several steps in defined order: array fabrication, target preparation, hybridization, washing, image capture, and data analysis.[12]

Array fabrication or construction of DNA microarrays (Fig. 1): it is a several-step procedure which involves obtaining the DNA sequences, design of oligonucleotides or primers for generating probe DNA, selection and preparation of suitable glass surface and depositing the probe DNA on its surface. Production of an array begins with the selection of the probes to be printed on it. In most cases, these are chosen directly from databases such as GenBank,[13] dbEST,[14] and UniGene,[15] which are the resource backbones of the array technologies.[16] Additionally, full-length cDNAs (complementary DNA), collections of expressed sequence tags (ESTs) or partially sequenced cDNAs or randomly chosen cDNAs/DNAs from any library of interest can be used. The probes DNA are oligonucleotides or polymerase chain reaction (PCR) products amplified from an individual clone using specific primers or universal primers if all genes were cloned in the same vector.[12]

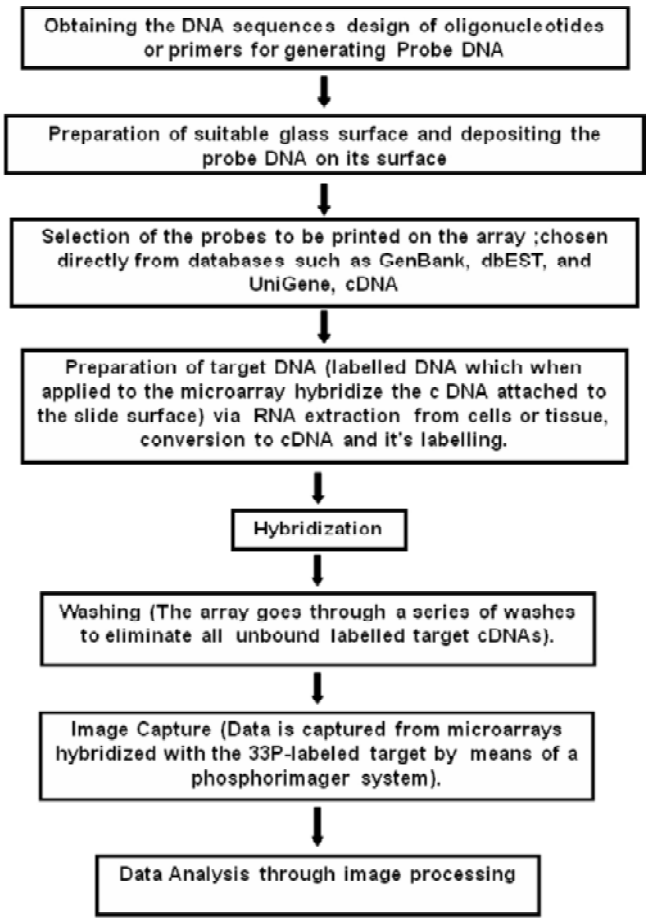

Figure 1: Flow chart showing steps of construction microarray

Preparation of target DNA: Target DNA is described as the labelled DNA which when applied to the microarray and hybridize the c DNA attached to the slide surface. The target DNA is usually created by reverse transcription of RNA into cDNA in such a way that a fluorescent nucleotide is incorporated. Preparation of target DNA includes RNA extraction from cells or tissue, conversion to cDNA and it's labelling.

Hybridization: it is the process of incubating the labelled target DNA with the probe DNA bound to the microarray substrate. Fluorescent target DNA hybridized to cDNA on the slide, and the number of immobilized fluorescence or radioactivity can be determined. Hybridization of the labelled probe is ideally linear (i.e. proportional to the amount of the probe) and sensitive (low abundance genes are detected) and specific (targets hybridize only to the desired genes in the complex probe mixture).[12]

Washing: After hybridization, the array goes through a series of washes to eliminate all unbound labelled target cDNAs. Washing breaks unstable binding of target and probe cDNAs, which may be the result of cross-hybridization. Washing is important, as incomplete washing causes deposition of salts or fluorescent materials that may cover the array partly or completely.

Image Capture: Data is captured from microarrays hybridized with the 33P-labeled target by means of a phosphorimager system. Microarray reader or scanners capture the signal intensities of all the spots on the glass slide with fluorescently labelled target. They are computer-controlled inverted scanning fluorescent microscope with a double or multiple lasers illumination system. Laser stimulates the microarrays hybridized with fluorescent targets. The emitted fluorescence is then captured by a CCD camera, non-confocal or confocal laser scanner.

Data analysis: Image processing is the first step of data analysis. Images generated by a microarray scanner represent the raw data of any microarray experiment. Computer algorithms convert the image into the numerical information that quantifies gene abundance. The second transformation, called normalization, removes non-biological influences on biological data, including unequal quantities of starting RNA, differences in labeling or detection efficiency. The third and last part of data analysis is one of the core goals of microarray analysis: to identify which gene is differentially expressed.[12]

TYPES OF ARRAYS

Three types of DNA microarrays in common use are: (1) microarrays where the probes are synthesized in-situ directly onto the surface of the chip (e.g., GeneChips), (2) double-stranded DNA microarrays and (3) oligonucleotide DNA microarrays. The latter two types are independently

synthesized probes that can be "printed" on special glass slides.[17]

The most noticeable microarrays with in-situ synthesized probes are the GeneChips made by Affymetrix (Santa Clara, CA, USA), produced by chemical synthesis of the oligonucleotides directly on the coated quartz surface of the array.[18-21] This technology allows very high feature densities, of about 400,000 features on a commercial array,[22,23] hence, called as high-density oligonucleotide arrays. GeneChips are created in a unique photolithographic process similar to the methods used for production of microelectronics chips in combination with chemical reactions established for combinatorial chemistry.[24]

A thin layer of a light-sensitive compound is coated over a quartz wafer that prevents the covalent coupling of an activated nucleotide. Exposure to light causes the removal of the chemical protection groups from the surface. Subsequently applied reactive derivates of single nucleotides can then be coupled. The attached nucleotides again carry a light-sensitive security group that has to be removed by illumination before coupling the next nucleotide. Lithographic masks are used to block or transmit light onto specific features, thereby determining the nucleotides to be coupled to the developing oligonucleotides. In repeated cycles of masking, light exposure, and coupling, oligonucleotides of 25 residues' length are synthesized on the chip surface. As the specificity of a probe of 25 nucleotides may not be high enough, each probe ("match") is accompanied by a mismatched probe which is a negative control along with a single differing base in the middle of the probe. Performance of probe and mismatch probe can therefore, be used to detect and eliminate cross-hybridization. Probe and mismatch probe are called a probe pair. Usually, a single gene is represented by 11 to 15 probe set. The very high feature density in this microarray enables the high number of controls.[17]

In printed microarrays, the probes are first synthesized independently and then with a microarray-spotter, spotted on the surface of the array.[25] There are two different technologies: contact printer and the non-contact printer. Contact printers spot the features by split or channelled pins, flat-tipped pins, and "pin and ring" types of pins.[26] All the pins are firstly dipped into a solution of the probe, and then onto the slide surface, thereby placing small droplets of less than 1 to few nanoliters on the surface of the slide. This results in features of 100 – 150 μm in diameter, with their centres positioned in the grid of 190 - 250 μm. Before next spotting, the pins are washed automatically. Hundreds of such pins can be assembled in so-called "print-heads". The features printed by one pen of a print-head are sometimes referred to as pen group or subgrid.[17]

Noncontact printers use bubble jet[27] or inkjet[28] technology analogous to computer printers. They shoot small droplets containing the probe on the surface of the chip but may result in missing spots because of cross-contamination and clogging of the capillaries. The foremost advantage of printed microarrays is their standardized dimension. Double-stranded DNA and oligonucleotides DNA are two major probe types for DNA microarray printers.

Double-stranded DNA microarray: Double-stranded DNA results from polymerase chain reaction (PCR) amplification. A 200- to 800-bp lengths of amplified DNA is recommended, but larger fragments of up to 1.3-kb length also work.[17,29] In typical microarray design, each probe DNA corresponds to one gene. In prokaryotes, two specific primers together with chromosomal DNA as template are used to amplify genes or parts thereof. However, there are also numerous variations to this strategy. Such clones allow the amplification of parts of the genes with only one specific primer. This way, about 80% of the genes of a typical prokaryote are possible to amplify with only one specific primer, thus reducing the costs greatly. Moreover, it increases the accuracy because only the correct combination of primers and template results in a PCR product of the expected size. Nevertheless, the generation of whole-genome DNA microarrays by high-throughput PCR amplification is a very laborious and logistically demanding process. Extensive quality control by gel electrophoresis, purification of products, and repetition of dropout reactions are necessary. The double-stranded DNA is printed on positively charged coated slides.[30] In most cases, they are coated with poly-L-lysine or 3-aminopropyltrimethoxysilane (APS). Spotting is usually made with a 1:1 solution of purified products of PCR at a final concentration of 0.2 to 1 mg/ml and dimethylsulfoxide (DMSO).[25] The DNA is bound to the surface of the slide with negatively charged phosphate backbone of a nucleic acid by electrostatic interaction.[31] This also helps to separate the two strands of a double-stranded DNA. Further baking at 80°C or UV cross-linking may introduce covalent bonds of thymine residues in the DNA to the amino groups of surface of the slide.[32,33] An additional blocking step is required to prevent nonspecific interaction of the surface of the slide to the target DNA especially for arrays printed on poly-L-lysine coated slides. This "post-processing" is done by incubating the slides in a freshly prepared succinic anhydride solution that readily reacts with the amino groups from poly-L-lysine.[34] The coated slides are commercially available from many suppliers, but can also be prepared in the laboratory by intense cleaning of special microscopic slides and dipping them in poly-L-lysine solution.

Higher hybridization specificity, sensitivity, and low cost are advantages of DNA microarrays made from spotting double-stranded DNA. They are essential whenever the sequence of the organism under study is not available. The laborious production of PCR products and the errors in probe identity that may result from mistakes during their production, is the greatest drawback, and has been reported to have a wrong

identity in commercial cDNA microarrays in 1 - 5% of probes.[35]

Oligonucleotide DNA microarrays: Using synthetic oligonucleotides as probe is an alternative to double-stranded DNA,[36,37] because they need much fewer logistics and are less error-prone due to automatic manufacturing of the oligonucleotides by the suppliers and their well-documented delivery in microtiter plates. By using longer probes with a length of 50 to 70 bp, their initial drawback of lower specificity and sensitivity due to short oligonucleotides of 25-bp length have been overcome.[38-40] However, this short probe length is a major benefit of oligoprobes as it allows to focus transcription analysis to intergenic regions or checking of very small open reading frames transcription. However, oligonucleotides as probes require a careful design.[41-43] All calculated melting points must fall into a temperature range of 5°C, and self-homology has to be avoided. Because of their short size, oligonucleotides are usually attached to the slide surface by covalent coupling. Otherwise, a significant amount of probes would be lost from the array surface during hybridization and washing. A large multiplicity of chemical reactions has been proposed to achieve covalent coupling, but the majority of slides used for spotting oligonucleotides are coated with compounds providing aldehyde or epoxy functional groups. To achieve covalent linkage, oligonucleotides used are with alterations at the 5' or 3' end; that increases the probe sequence availability for hybridization to the target because it is not fixed to the surface by its backbone or bases. An additional increase in sensitivity can be obtained by inserting spacer molecules between the oligonucleotide and the slide surface.[44-46] The most common modification of oligonucleotides is a 5' amino group.[47] It offers a high flexibility in the choice of slide chemistry: aldehyde and epoxy groups react specifically readily with the primary amino group. Modified oligonucleotides are usually spotted at a concentration of 10–30 μM. The conditions have to be accustomed so that the coupling can proceed. Finally, the functional groups of the array which are not part of a feature should be blocked, similar to arrays made from double-stranded DNA probes. This can be completed, by incubating the slides in the presence of low molecular primary amines. Printed microarrays can be stored for months when kept under completely dry conditions in desiccators and are protected from light.[48]

APPLICATION OF MICROARRAYS

Conventional microbiological approaches rely on cultivation, are time-consuming, require specialized and complex growth media, capture only a minor fraction of the oral microorganisms, and do not offer in vivo data of gene expression during infection and subsequent disease. Although informative, labor-insensitive molecular techniques e.g., clone-libraries, quantitative-PCR, as well as fluorescent in situ hybridization studies, are impractical for routine patient monitoring. Whereas microarray technique, is highly parallel, and highly sensitive instruments that provide high-fidelity data in a high-throughput format to characterize the complex communities of human oral cavity.[1]

In general, microarray technology is used for transcriptional profiling, genome-wide network analysis, analysis of mutants and transgenics, gene copy number, resequencing, genotyping, single nucleotide polymorphism, DNA-protein interaction, gene discovery or mapping. Recent studies have reported their use in gene regulation studies, drug discovery and toxicology,[12] identification of new cell lines and in protein and compound arrays. Since its inception, microarray analysis, provide information about the up-regulated/ON and downregulated/OFF genes under certain environment,[1] as well as expediting comprehensive countenance analyses of an enormous number of distinct genes.[49] A common use of microarray has been the measurement of gene expression, from characterizing cells and processes to clinical use such as tumor classification.[7,50-54] Microarrays could also be utilised to monitor and characterize the trafficking of cellular RNA through p-bodies (complex of protein and RNA) complex,[55] to examine aneuploidy and changes in loci copy number in a variety of cell types,[56] to examine the development of replication forks as they copy the genome,[57] for genome-wide screens of RNA modifying enzymes,[7,58] and to identify functional elements in the genome.[7]

The dominant applications of microarrays have been in measuring gene expression in different situations. This includes the studying of diseased versus healthy tissues, profiling tumors and predicting outcomes, studying gene regulation during development, and following the stimulation of cells in vitro. In addition, scientists are conducting profiling research that may lead to the use of microarrays in personalized medicine, in molecular diagnosis of disease, and in predicting drug efficacy and toxicity in different individuals.[2,59] The combined use of microarray techniques with the computer programs for the differential analysis of gene expression microarray experiments provided a set of candidate genes that may help as innovative therapeutic intervention points and enhanced diagnostic and screening methods for high-risk individuals.[2] Additional uses of microarrays include the examination of pathogenic genetic variations and single nucleotide polymorphisms (SNPs) detection. Microarrays provide high promises for advancements in oral cavity biology for dentists in 21st century, specifically useful for the diagnosis of microorganisms in the oral cavity.[49] Li *et al*.[60] in 2004 used high-density oligonucleotide microarrays for salivary profile transcripts of head and neck cancer patients. In the near future, the use of microarray-based miniature detectors with heightened sensitivity for percipience between perfectly matched hybridizations and cross-hybridization events in the dental field would help dentists and clinicians to detect

microbial lookouts in the oral cavity. It will also provide enhancements in diagnosis, prevention, and monitoring procedures, which will lead to improved management of patients' oral care.[1]

CONCLUSION

Microarrays made important contributions to both the basic and applied research with promise to change future practice of medicine, as well as dentistry. However, as Ingen *et al*.[59] in 2002 quoted, "although, nearly a decade has passed since first microarrays were produced, and yet we are just beginning to perceive what can be achieved with this technology," its solicitation in all disciplines of oral as well as systemic health is under development. Future will allow researchers to provide improvements in diagnosis, prevention and various techniques to provide better health management of the patients, based on randomized clinical trials.

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