Chapter 12

DNA Microarray-Based Diagnostics

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Abstract

The DNA microarray technology is currently a useful biomedical tool which has been developed for a variety of diagnostic applications. However, the development pathway has not been smooth and the technology has faced some challenges. The reliability of the microarray data and also the clinical utility of the results in the early days were criticized. These criticisms added to the severe competition from other techniques, such as next-generation sequencing (NGS), impacting the growth of microarray-based tests in the molecular diagnostic market.

Thanks to the advances in the underlying technologies as well as the tremendous effort offered by the research community and commercial vendors, these challenges have mostly been addressed. Nowadays, the microarray platform has achieved sufficient standardization and method validation as well as efficient probe printing, liquid handling and signal visualization. Integration of various steps of the microarray assay into a harmonized and miniaturized handheld lab-on-a-chip (LOC) device has been a goal for the microarray community. In this respect, notable progress has been achieved in coupling the DNA microarray with the liquid manipulation microsystem as well as the supporting subsystem that will generate the stand-alone LOC device.

In this chapter, we discuss the major challenges that microarray technology has faced in its almost two decades of development and also describe the solutions to overcome the challenges. In addition, we review the advancements of the technology, especially the progress toward developing the LOC devices for DNA diagnostic applications.

Key words DNA microarray, Stand-alone lab-on-a-chip (LOC) device, Diagnostic tool, Microfluidics, Label-free detection, Nanoarrays

1 Overview

The DNA microarray has achieved significant progress in both application and technology ever since it was first introduced in 1995 by Schena et al. [1]. This first microarray was fabricated by spotting or printing various complementary DNAs (cDNAs) on a glass microscope slide via a robotic printer and the microarray was used to monitor the differential expression of many genes in parallel. There are three major applications of the microarrays. First, the microarray platform, especially in the early years of developments,

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has been used to obtain clinically relevant information from the gene expression levels [2]. For example, the microarray data were used to differentiate between cancer subtypes, to provide prognostic information (e.g., likelihood of recurrence or metastasis) and predictive information (e.g., efficacy of chemotherapy). Second, the microarrays have been developed for genotyping to characterize the DNA (or RNA) in order to detect human gene mutations (or characterize viral pathogens). While simple genotyping arrays consist of hundreds of features or spots, complex arrays utilize thousands of features to investigate mutations in many genes or to characterize multiple sequences in the pathogen genomes. Third, the microarrays are used to conduct the array-based comparative genomic hybridization (array-CGH) which provides a high-resolution tool for screening copy number variations (CNV) in the whole genome and offers several advantages over classical techniques [3]. In addition to the various novel applications, the microarray platforms experienced many technical advances after the commercial vendors took over the developments. For instance, the probes to be immobilized on the microarrays have shifted from cDNA to short oligonucleotides, and they were either pre-synthesized or synthesized in situ. These oligonucleotides demonstrated a higher specificity than cDNA probes. Glass is still the predominant substrate used in the microarray platform, but materials such as silicon and polymers have also been used as the microarray substrate. In terms of signal transduction to generate the microarray data, label-free detection techniques have also been developed [4].

Although the microarray technology should have a high potential for clinical applications, it has not experienced a smooth path of development. There are numerous challenges, more on biostatistics than on technical issues. One challenge is that the microarray data have notoriously been considered as being "noisy" [5]. The reproducibility of the data and the validity of the data interpretation reported by prominent microarray studies have been criticized because of a lack of appropriate standardization, adequate quality control measures and reliable data processing [6]. The uncertainty about the validity of the microarray data interpretation hindered the approval of array-based clinical tests by regulatory organizations as well as the subsequent adoption of the tests by clinical communities. The concerns about the validity of the microarray data interpretation is more serious in applications such as the introduction of new biomarkers (e.g., expression profiling), rather than in applications such as genotyping which are dealing with preexisting biomarkers.

Another challenge of microarray-based tests is the advent of the competitive PCR-based and sequencing-based tests. For instance, simple microarray tests, when only a few genes are being

monitored or a limited number of mutations are being interrogated, have to compete with the well-known PCR-based tests. On the other hand, complex microarray tests, which provide large amounts of information unattainable by PCR-based techniques, are facing a strong competition with the newly emerged next-generation sequencing (NGS) techniques. They generate detailed information about the whole genome with the prices that have been tremendously lowered in recent years [7].

This chapter is dedicated to discuss the major challenges that the microarray technology has faced in the pathway of its growth since its inception. We also highlight the progress that has been achieved by the research and commercial communities to overcome the obstacles.

2 Reliability of Microarray Data

Since the advent of the DNA microarray technology, some concerns have been raised regarding the reliability and reproducibility of the microarray data [6, 8]. A meta-analysis was performed on the reproducibility of the data of seven large scale studies on cancer prognosis that used microarray-based expression profiling [8]. Surprisingly, in five of these studies the reported data were not reproducible. The analysis of the other two studies provided much weaker prognostic information than given by the original data [8]. Following the awareness about the shortage of standardization measures, the scientific community put much effort in preparing appropriate standards, controls and tools [9, 10]. Aiming to provide a basis for reporting the microarray results, the standard called MIAME (minimum information about a microarray experiment) was proposed [9], which ensured that the microarray data can be easily interpreted and independently verified. Commercial vendors of the microarray platforms improved their technologies over the years, and they also set up a series of quality control measures to enhance the reproducibility and accuracy of the data produced by their products. Together with the regulatory agencies, the vendors started the MAQC (Microarray quality control) project [10], which established thresholds and metrics for inter-platform comparison of microarray data.

In microarray analysis, especially in gene expression profiling where a massive amount of information is commonly produced, it is critical to provide the biological interpretation with statistical significance. In order to decide if a gene is outcome-related, the expression level of the gene in the patient sample is usually compared with the one from a normal sample, and a fold-change and clustering analysis are used to provide the biological interpretations, e.g., class comparison or prediction of the disease in cancer

patients [6]. However, the expression levels of the genes naturally vary between different individuals and between different samples from the same individual [8]. Hence, a simple fold-change statistic does not account for the variability across specimens, leading to false positive outcomes. For instance, in 2007, Dupuy and Simon who reviewed 23 studies that reported results of outcome-related gene-finding analyses found out false-positive results in nine of them [5]. Because of the recent advances in bioinformatics, valid data analyses are currently available. The so-called supervised data interpretation methods are able to make distinctions among the specimens based on predefined information and to create valid information for clinical decision-making [6].

3 Microarrays Integrated with the LOC Devices

Integration of various steps of microarray assay in a miniaturized, portable and stand-alone lab-on-a-chip (LOC) device is a crucial requirement for a variety of applications, especially point-of-care (POC) diagnostics [11]. Current microarray technologies use separate instruments for sample preparation, DNA hybridization, signal visualization and data interpretation. Moreover, some of these components such as the fluorescent scanners used for signal visualization are bulky instrument that are only available in well-equipped laboratories. The development in sampling and detection technologies certainly accelerates the process of integration. For instance, some of the sample preparation steps for sample labeling can be avoided when label-free detection approaches are used [12–14]. Miniaturization of the microarray spots also alleviates the need of fluorescent scanning and so no bulky fluorescent scanners are required. More importantly, with the aid of microfluidic network, all steps of the microarray test can be integrated in a single miniaturized device. In the following section, we will present the advances and challenges in developing the technologies required for the microarray tests to be integrated in a stand-alone LOC device.

3.1 DNA Microarrays Combined with Microfluidic Networks

The developments in the microlithography techniques have enabled microfluidics which is used to create LOC devices [15, 16]. Coupling the microfluidic operations with microarray assays potentially adds precious value to them. One obvious benefit of microfluidic liquid-handling is the reduced sample and reagent consumption due to small micrometer-sized channels. In addition, the highly efficient and controllable pressure-driven flow for liquid handling and delivery in these microchannels allows for integration of different steps of microarray assays that are essential to implement the portable LOC devices [17]. More importantly, the target molecules in the samples are delivered to the probe spots in the

microarrays using the convective flow, in additional to diffusion, and thereby reducing the hybridization times from hours to minutes. Furthermore, the benefit of the microfluidic microarray LOC device is in its potential on high sample throughput as well as sufficient number of probes. Conventional microarray experiments usually allow 1–10 samples to be applied on one chip [18], and so replicate analysis will require multiple chips. As discussed in **Subheading 2**, one of the challenges of DNA microarrays is the inevitable variations among samples [6], and these variations make replicate analysis of several samples necessary, and therefore the multi-sample analysis capability of the microfluidic microarray chips is highly valuable.

There are two ways to conduct the microarray analysis in the microfluidic chips, either in the microfluidic chambers or in the microfluidic channels [19–21]. First, large microfluidic chambers are used to enclose the area that is pin-spotted with arrayed probes, where the sample DNA molecules are hybridized with the probe molecules [22]. These chambers are compatible with both low-density and high-density microarrays, but it is always a challenge to design how the liquid will flow uniformly over the large chamber in such a way to achieve an equally distributed liquid movement across the arrays. Second, the microfluidic channels are used and they provided a better flow control of target solutions over the probe arrays. Various microfluidic chips containing straight and serpentine microchannels have been implemented, mainly for low-density microarrays [23]. In these cases, the pin-spotted probe regions are usually enclosed along the channel length of the microchips. Especially in the second way of conducting the microarray experiments, the microfluidic operations benefit the effective interactions between the target molecules in the sample solutions and the probe molecules immobilized on the channel surfaces due to the use of a dynamic flow.

In addition to benefit effective sample delivery, the dynamic microfluidic flow is used to facilitate the probe printing on the microarray surface in a uniform manner. The performance of hybridization in the microarray assay is heavily influenced by the morphology of the printed spots on the chip surface. However, since the probe solutions are exposed to air in the conventional probe-spotting method using pins, the solutions are subject to various problems, such as splashing, uneven evaporation and cross contamination [24], leading to unacceptable spot morphology. Even worse, during the blocking and cleaning procedures after probe-spotting, the unreacted probe molecules could diffuse away from the spot locations and smear to form comet-like spots [16, 25]. Furthermore, since the dynamic-flow hybridization is to be used with the spotted microarray, additional apparatus such as steel clamps must be used to ensure that the entire hybridization microchannel is well sealed and aligned to the probe rows [16].

These problems would be resolved by using the microchannel network for microprinting the probes, resulting in high homogeneity of the probe regions printed on the microfluidic microarrays. For instance, Wang et al. used a network of microchannels in two steps: first for probe printing and second for DNA hybridization, producing the 2D microarrays [20]. In the first step of this method, called the 2-step intersection approach, the probe solutions flowed in the horizontal microchannels, in the first polydimethylsiloxane (PDMS) slab sealed with a glass chip, in order to print an array of horizontal probe lines on a glass chip surface. In the second step, the target solutions flowed in the vertical channels, in the second PDMS slab sealed with the same glass chip, in order to hybridize with the spotted probes at the intersections between the vertical microchannels and the horizontal probe lines. The 2D microfluidic microarray is well suited for parallel sample hybridizations and, unlike the low-density DNA microarray spots printed by pins, the use of long and narrow probe line in microfluidic microarrays alleviates the need of time-consuming alignment between the hybridization channels and the printed probes.

The 2D microarrays can be used for many diagnostic applications. Since in many of these applications that deal with many samples, once a relatively small number of gene mutations or single nucleotide polymorphisms (SNPs) are identified, low-density microarrays can be employed to screen these mutations across many patient samples. This low-density microarray approach has been demonstrated to be reliable, cost-effective, and fast in data analysis and interpretation [26–28]. In order to perform SNP analysis for the KRAS mutation on the chip, Sedighi et al. replaced the regular free DNA targets by the gold nanoparticle-loaded targets to enhance the specificity of DNA hybridization reactions on the chip surface [28, 29].

So far, the dynamic flow used in microfluidic microarray chips has been achieved by pressure. An alternative to the pressure-driven flow is to achieve liquid pumping by centrifugal forces. Centrifugal pumping used for dynamic liquid flow has several advantages such as easy implementation and insensitivity to the physiochemical properties of the liquid. Using centrifugal force, the liquid can be transferred in a parallel manner in multiple channels of a disk-like chip by spinning it. Furthermore, the implementation of centrifugal force by disk spinning is compatible with the CD/DVD technology and its related industries which have been well developed. In most of the reported applications that utilize the centrifugal platform, only the radial channels are used for liquid handling and delivery. For instance, Bin et al. reported a CD-like device capable of generating the flow of DNA samples within the twelve PDMS microchannels for DNA sample delivery to the 1D microarray (Fig. 1), with the sample hybridization time reduced to 15 min and the sample volume as low as 1.5 μL [30]. However, this format for

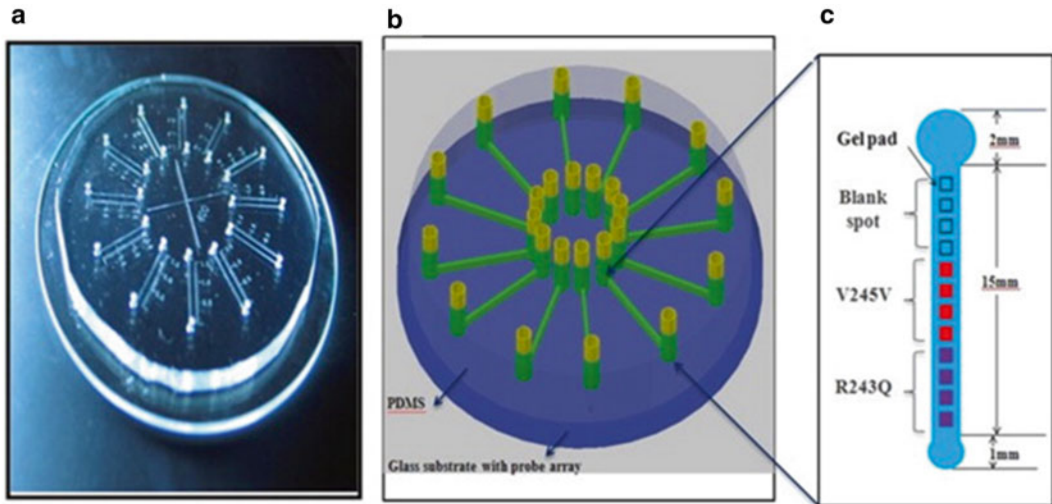

Fig. 1 (**a**) The photograph of the PDMS-glass CD-like chip. (**b**) The two-layer structure of the microfluidic chip including a top PDMS slab containing 12 hybridization microchannels, and a bottom glass disk with the immobilized 1D microarrays. (**c**) The DNA arrays for phenylketonuria (PKU) screening for R243Q and V245V mutations as well as the negative control probes (reproduced from ref [27] with permission from Elsevier

centrifugal liquid delivery has a design limitation because there is not enough space to accommodate the multiple fluid structures in the radial format [11]. For example, if the centrifugal platform is built on a 92-mm CD with a 15-mm center spindle hole, the maximum limit of the length of a radial microchannel is 38.5 mm. For such a short microchannel, the capillary effect may dominate the liquid flow and the flow velocity cannot be easily controlled. More importantly, by using centrifugal pumping only once in the radial direction, the intersection method cannot be applied to generate the 2D microfluidic microarray.

To integrate centrifugal pumping with the 2D microarray, Peng et al. exploited the centrifugal force twice based on the sequential use of two chips with specially designed channels in order to create a 2D microarray [31–33]. As shown in Fig. 2, in addition to the radial microchannels, which were used for probe printing, spiral microchannels were employed to implement target hybridization by the intersection method. In this method, a PDMS slab containing radial microchannels was first sealed against the glass disk and used for printing the radial probe line arrays on the disk. After the first slab was removed, a second PDMS slab that consisted of the spiral microchannels was sealed against the same disk, and DNA hybridization occurred at the intersections between the spiral microchannels and the radial probe lines. Dynamic target delivery facilitated by the centrifugal force can be conveniently controlled and synchronized [31–33]. The 2D microarrays generated using CD microfluidics also demonstrated a high sensitivity

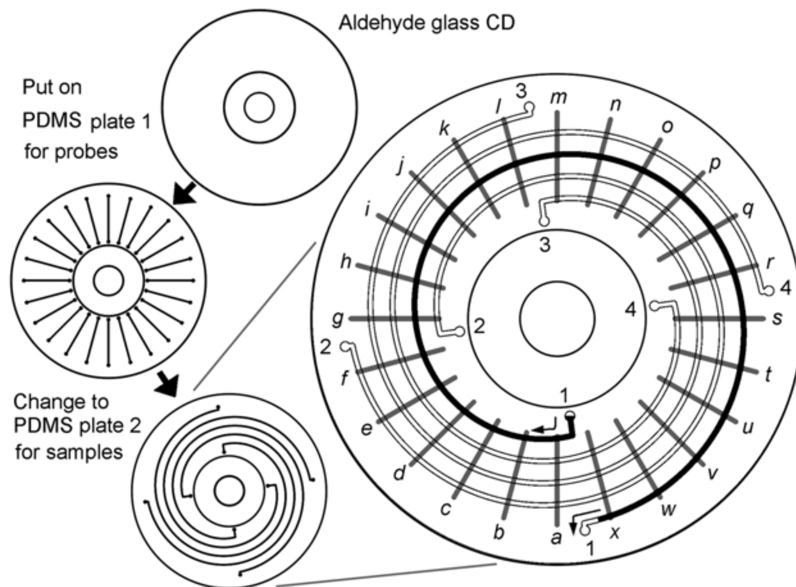

Fig. 2 2D microfluidic microarray analysis using the intersection method. Printing of DNA probes using the radial channel chip 1, with 24 DNA probe lines printed (*solid radial lines* marked by a–x). DNA Hybridization using the spiral channel chip 2, with four samples flowing through the four spiral channels (hollow spiral channels marked by 1–4). Liquid flow in spiral channel 1 was indicated by *black* color and the *two arrows*. Hybridizations occur at the intersections of the spiral channels and radial probe lines (reproduced from ref. [28] with permission from Elsevier)

and specificity for DNA analysis [34, 35]. So far, a higher spot density (384 × 384) has been achieved for the high-throughput microarray analysis on a 92-mm CD-like glass disk [36].

3.2 Advances in Detection Techniques

Fluorescence detection is commonly used for DNA microarray assays, in which the sensitivity and stability of the detection method have vastly improved over the years due to the discovery of new fluorescent dyes and more effective labeling techniques [37]. However, bulky fluorescent scanners are still required to achieve a high sensitivity and resolution, which is a limitation to the miniaturization requirement for LOC device developments. The efficiency of target labeling and fluorescence quenching of the dye also affects the reproducibility of the results [38]. In addition, fluorescent labeling of the target molecules adds complications and cost to the assays, and so several approaches have recently been developed in order to avoid target labeling [38].

One novel approach is the use of molecular beacon (MB) that takes advantage of both the sensitivity of fluorescence detection and convenience of no target labeling. However, the MB probes are still labeled [39], and they are single-stranded nucleic acids that retain a stem-and-loop structure and keep a pair of fluorophore-quencher

at both ends of the stem strand in close proximity and thus the fluorescence emission of the fluorophore is quenched. In the presence of a non-labeled target molecule, the loop-region of MB probe hybridizes to the target, while the stem opens up and fluorescence of the fluorophore occurs when the quencher moves away [39].

Other novel detection techniques, based on the optical, electrochemical and microwave properties of the target molecules, have also been developed in order to alleviate the need of target labeling [38–43]. Surface plasmon resonance imaging (SPRi) is an attractive detection approach for microarrays because none of the targets or probes requires labeling, and thus this is a true label-free detection [40]. Moreover, Özkumur et al. developed the spectral reflectance imaging biosensor (SRIB) for high-throughput analysis of SNPs on a glass microarray chip [43]. This technique, which is based on optical interferometry, has allowed the single-nucleotide mismatched target oligonucleotides to be distinguished from perfectly matched ones, through dynamic data acquisition during the washing step using a low ionic concentration buffer [43].

Furthermore, an electrochemical technique using multiwalled carbon nanotubes (MWNT) nanoelectrode arrays was developed by Koehny et al. to detect unlabeled PCR amplicons [41]. In this technique, with the aid of $Ru(bpy)_3^{2+}$, the guanine bases in the DNA targets serve as the signal transduction moieties, providing an amplified anodic current associated with the oxidation of guanine groups at the nanoelectrode surface. The abundance of guanine bases in the target strands led to a high sensitivity and low detection limit, i.e., less than ~1000 target amplicons on a microspot are detectable. Another label-free detection is near-field scanning microwave microscopy (NSMM), which has been used by Lee et al. for detection of both DNA and RNA molecules [42]. NSMM monitors the microwave reflectance, which is dependent on the length and surface coverage of the nucleic acid strands, as well as on the hybridization state of the molecules (e.g., unhybridized single-stranded probe vs. hybridized double-stranded). The NSMM technique has demonstrated an acceptable resolution (potentially less than 50 μm) and a sensitivity comparable to fluorescent detection [42].

3.3 Miniaturization of Microarray Features

A major challenge in developing portable microarray devices is the need of large-format fluorescent scan. It is because the fluorescence detector, which utilizes a high numerical-aperture (NA) microscopic objective in order to detect the weakly fluorescent microarray spots, has a narrow field of view and so covers only a few microarray spots. Miniaturization of the microarray features in such a way that the whole array would be visible in the field of view of the high NA objectives would render the scanner unnecessary. Other than alleviating the need for a scanner, miniaturized arrays

also favor fast mass and heat transport, and therefore reduce the assay time [44].

Creating an array with sub-micrometer features requires very accurate probe printing techniques with nanometer resolutions. Among different nanoprinting methods, the scanning probe microscopy (SPM)–based technique received a high level of attention in this regard. The SPM-based technique produces probe printing in a high positioning precision and also its non-vacuum operational condition is compatible with biomolecules [45]. As an example of this technique, Demers et al. used dip-pen nanolithography (DPN) to directly couple hexanethiol-modified oligonucleotides on the gold surface, and acrylamide-modified oligonucleotides on silica substrates [46]. They managed to reduce the size of the microarray features to 100 nm which allows an array containing ~100,000 features to be generated in an area compatible with the size of a typical AFM scanner [46]. Despite the extremely high resolution of DPN, this technique is intrinsically serial, and thus a significant amount of time must be allocated in order to generate the microarrays [47]. Nanoimprint lithography (NIL) is another approach employed for the reduction of feature dimensions in microarrays. In NIL, probe oligonucleotides were either synthesized in-situ on the chemically modified nanostructures created on a polymer surface [48], or physically tethered to the surface of nanostructures before they were delivered (or stamped) on the substrate [47]. For instance, two research groups independently developed a technique to replicate the whole DNA array in a single cycle [49, 50]. In this technique, a first master substrate made of oligonucleotide probe features was immersed in a solution containing the complementary target DNAs. Afterwards, a second substrate was brought into contact with the first substrate to adsorb the target DNAs, thus replicating the features on the second substrate. Such an effective nanostamping method was able to reproduce DNA arrays with features as small as 14 nm, with the spacing of 77 nm [51]. Advances in miniaturization of the size of the printed probes, in the nanometer scale, are significant steps moving toward the development of portable microarray platforms. It also makes the microarray test cheaper by avoiding the scanner and by reducing the amount of sample biomolecules.

3.4 Advances in Integration of LOC Devices

Many LOC microdevices have been developed to perform individual steps for DNA microarray assays. However, integration of these devices to give a POC diagnostic system in an efficient manner remains a challenge. All steps in an integrated system such as liquid handling, reagent metering, thermal and pressure control and signal transduction must be compatible with each other. Despite these challenges, great effort has been made by the researchers to develop the system that is able to perform the many steps of the microarray assay [52–58]. Anderson et al. reported one such system, which was

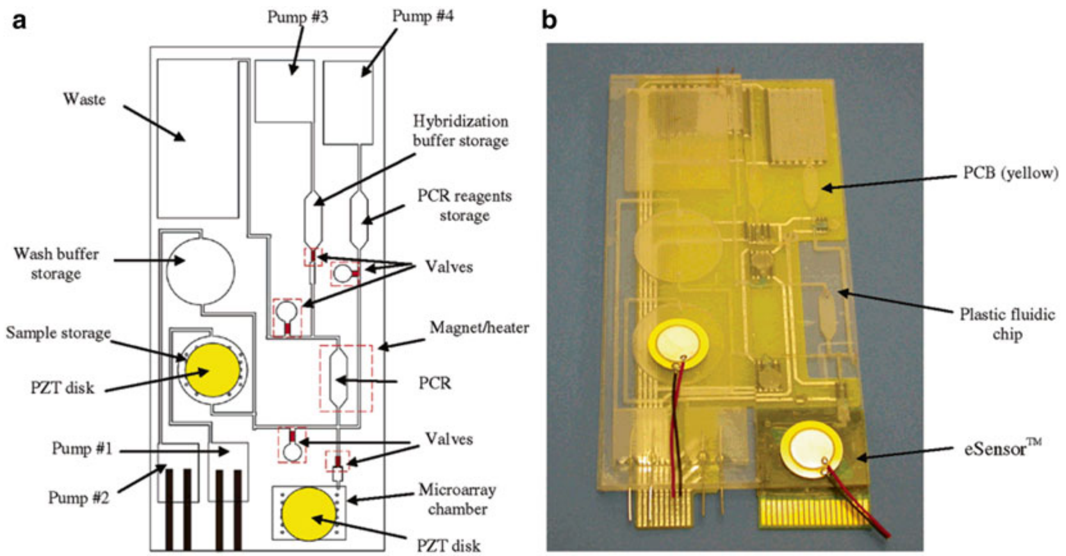

Fig. 3 The integrated microchip for DNA microarray detection. (**a**) Schematic and (**b**) photograph of the integrated device, which consists of a plastic fluidic chip, a printed circuit board (PCB), and an eSensor located in the microarray chamber. The two PZT disks are piezoelectric devices that assist in reagent mixing (reproduced from ref. [49] with permission from American Chemical Society)

capable of extracting and concentrating nucleic acids from aqueous samples, performing chemical amplification and serial enzymatic reactions (fragmentation, dephosphorylation, and labeling), metering and mixing, and microarray-based nucleic acid hybridization, for the detection of mutations in the HIV genome [52].

Liu et al. developed an integrated microchip for DNA microarray detection of bacterial pathogens in blood [53]. Their device consisted of a plastic chip, a printed circuit board (PCB), and an eSensor. The plastic chip included a mixing unit for cell capture, cell preconcentration, purification and lysis, a PCR unit for nucleic acid amplification, and a DNA microarray chamber for hybridization. Thiol-terminated DNA oligonucleotides were immobilized on the eSensor for electrochemical detection of hybridized target DNA [53] (Fig. 3). Liu et al. also integrated a DNA microarray platform, containing 12,000 features within a microfluidic cartridge, in order to automate the fluidic handling steps required for gene expression profiling assay [57]. Microarray hybridization and subsequent washing and labeling steps were all performed at the self-contained device [57].

Yeung et al. also developed an integrated microchip that was based on a multi-chamber design for multiplex pathogen identification [56] (Fig. 4). In their silicon-glass chip, the oligonucleotide probes were individually positioned at each indium tin oxide (ITO) electrode within the microfluidic chamber. Several microfluidic-controlled steps, which included thermal lysis, magnetic particle-based

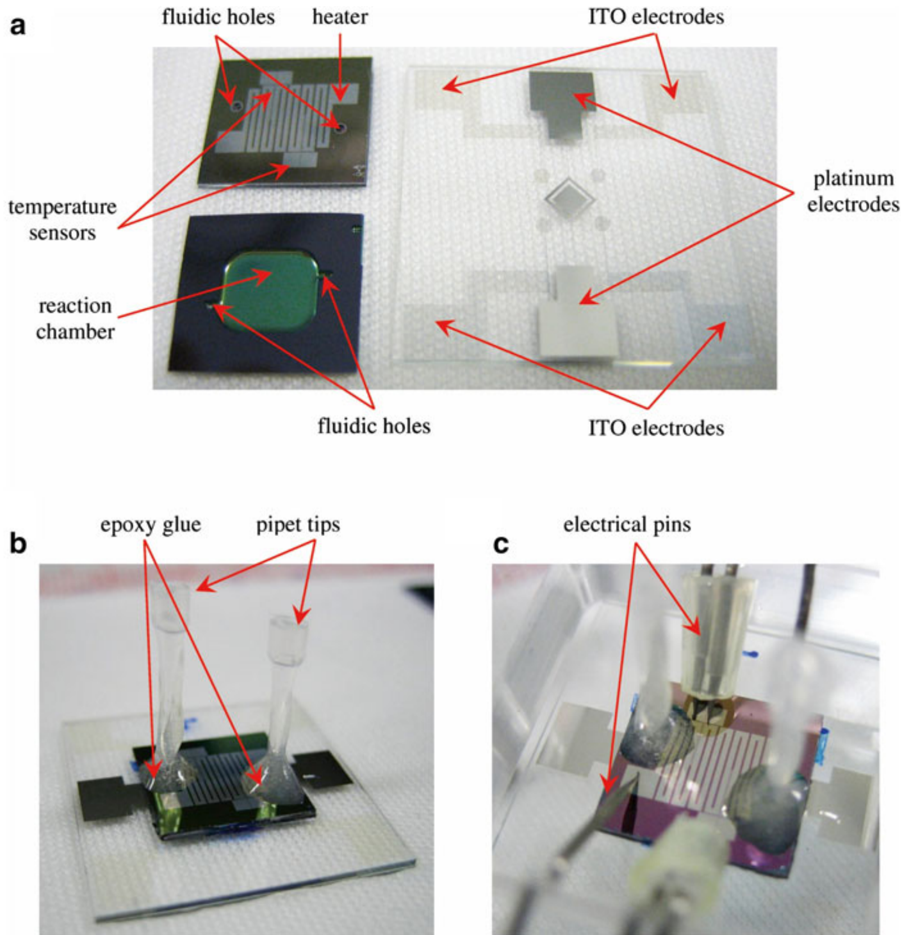

Fig. 4 The silicon-glass microchip for multiplex pathogen detection. (**a**) *Upper left*: top view of the silicon chip showing the fluidic holes along with thin-film platinum heater and temperature sensors; *lower left*: bottom view of the silicon chip showing the 8 mL reaction chamber and the through-hole for sample introduction; *right*: glass chip with patterned indium tin oxide (ITO) electrodes. (**b**) The assembled silicon-glass microchip, on which pipet tips were glued to the fluidic holes to form solution reservoirs. (**c**) Electrical connection of the contact pins to the ITO electrodes in the chip housed in a Plexiglas holder

isolation of the target genomes, asymmetric PCR, and electrochemical detection using silver-enhanced gold nanoparticles, were performed in the integrated device [56]. Liu et al. also integrated a DNA microarray platform, containing 12,000 features in with a microfluidic cartridge, in order to automate the fluidic handling steps required for gene expression profiling assay [57]. Microarray hybridization and subsequent washing and labeling steps were all performed at the self-contained device [57]. Choi et al. integrated an allele-specific PCR unit with a disposable DNA microarray chip for multiplex SNP detection. Convective flows, created by pneumatic micropumps, were used in this integrated system to accelerate

the hybridization process, resulting in the whole assay completed on-site in 100 min. In this assay, a miniaturized fluorescent scanner, instead of a conventional bulky one, was conducted for hybridization detection [58]. More work still needs to be achieved to further develop these LOC devices to become stand-alone (sample-in-answer-out) devices.

4 Commercialization and Clinical Use

The microarray technology has been slow to penetrate the molecular diagnostic market, i.e., only 10 % in 2010 [7]. The poor reproducibility of the microarray data, which is due to technical limitations or natural variations between different samples, has prevented the microarray tests to have sufficient robustness required for a diagnostic test. In order to receive regulatory approval and even clinical acceptance for expression profiling tests, they are required to demonstrate the result reliability and the correlation of their results to the clinical outcomes [59]. Gaining regulatory approval is difficult for gene expression profiling because it is commonly based on new research studies that correlate the clinical outcomes to the levels of expression of new genes, which are not predefined and well-known biomarkers. Furthermore, these tests face the clinical utility question: how do the microarray data improve the outcome of the patient? For example, what is the suitable type of treatment, and how is over-treatment in chemotherapy prevented [59]? MammaPrint (Agendia, Netherlands) was the first expression profiling test that received the FDA approval in 2007 for prognosis application in breast cancer [60]. MammaPrint, based on a research reported by Vijver et al. assesses the expression profile of a set of 70 cancer-related genes. However, the test suffers from a strong competition from the PCR-based Oncotype test (Genomic Health, USA), primarily because the latter is able to analyze the widely used FFPE (formalin-fixed paraffin-embedded) samples. To address this sample need, a new microarray-based test (Tissue of origin, Pathwork Diagnostics, USA), with the capability to operate on FFPE samples, has recently entered the market. Another competition is from next-generation sequencing techniques, which are well developed and their prices are no longer prohibitive [61]. These techniques (e.g., RNA-Seq) are more reliable and informative since they provide the sequence information without prior knowledge. Moreover, these sequencing techniques are convenient since they provide digital, instead of analog, data.

Unlike gene expression arrays, genotyping arrays have fewer obstacles to overcome in gaining regulatory approval as well as clinical acceptance. Genotyping, which aims to characterize previously established sequence variations among the genome, does not have to prove its clinical correlation and utility. Nevertheless, the genotyping

arrays are still required to be technically reliable and competitive in price. High-throughput genotyping arrays contain a significantly high numbers of features to investigate mutations in several genes or mixtures of pathogens and thus provide a huge amount of information. As an example, AmpliChip CYP450 obtained FDA approval in 2004 (the first microarray-based clinical test). The test uses 15,000 features on an Affymetrix platform to assess several types of variations in two genes, involved in the metabolism of many psychoactive drugs. On the other hand, low-throughput genotyping arrays use a fairly small number of features (up to few hundreds on a chip) for characterization of a pathogen or investigation of several SNP sites in a particular gene. The PapilloCheck test provided by Greiner Bio-One Company (Frickenhausen, Germany) is a fairly successful low-throughput genotyping test, which obtained the US regulatory approval in 2009. PapilloCheck utilizes an array of 140 oligonucleotides to determine the HPV (human papillomavirus) subtypes in cervical smear samples.

5 Future Perspectives of Microarray-Based Diagnostics

Recently, a number of LOC devices have been developed to integrate several steps of the microarray assay on a miniaturized platform. However, these devices still need to use the conventional methods for the signal detection [52]. The use of nanoarrays helps replace the bulky fluorescent scanner with the miniaturized fluorescence detector because the whole array can fit in the field of view of the detector. Other than fluorescence detection, label-free detection techniques will further simplify the future LOC devices. Different steps of the microarray assays are integrated in stand-alone LOC devices in a harmonious way, which make them capable of performing the sample-in-answer-out assays. These LOC devices exploit the microfluidic networks not only to connect the different compartments in the devices, but also to make the device faster, smaller and conveniently controlled.

DNA microarrays have faced fierce competition from next-generation sequencing (NGS) at the high end of throughput and from PCR-based techniques at the low end in the molecular diagnostic market. However, the cost of NGS assays, although not prohibitive anymore, are still more expensive than the ones offered by the microarray vendors. The presence of some unresolved difficulties in the sequencing techniques, like the necessity of multiplexing in RNA-sequencing as well as their extensive sample preparation and data interpretation needed for the techniques [62], will be in favor of the microarrays in the competition. On the other hand, since the microarray technique is more flexible in the sample matrix that it can process it is more familiar to the clinicians; the microarrays are in a better situation in competition with PCR-based

techniques. Because of the wealth of knowledge offered by the human genome, the size of the molecular diagnostic market has largely expanded in the past decade and is predicted to be doubled in 2017 [63]. More microarray-based diagnostic tests are currently gaining regulatory approvals and entering the market. According to a recent report, the microarray-based tests share the largest portion of the molecular diagnostic market with the PCR-based tests [63], and the market of the former type of test is expected to grow in the coming years [64].

6 Summary

Owing to technical quality issues, the microarray techniques face serious concerns and criticisms about the reproducibility of the data they provided as well as the reliability of the inferred biological interpretations. These shortcomings in the microarray techniques themselves as well as the harsh competitions from other molecular diagnostic techniques deter the microarrays to be as successful in the clinical market as they were in the research counterparts.

However, DNA microarray vendors and researchers managed to resolve many of those issues, which led to regaining the product confidence in the market. This is demonstrated as the growth of the number of microarray-based tests that receive regulatory approvals as well as positive foresights by business reports.

Meanwhile, much advancement has been achieved in the prerequisite technologies for developing portable and stand-alone LOC devices. Future DNA microarrays are expected to be faster, smaller and more accurate. Supporting systems, well-matched with the microarray platforms, will be developed and integrated into the complex stand-alone LOC devices. These LOC devices play a central role in personalized medicine in the future. Technical issues, observed in the early microarray platforms, are mostly resolved in the current platforms. New discoveries about the human genome, thereby increasing the depth of our knowledge about it, will initiate new clinical utilities for the DNA microarray. Enjoying all of these developments, DNA microarray will maintain a reasonable share in the fast growing molecular diagnostic market.

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