Microfluidic Device for Rapid (<15 min) Automated Microarray Hybridization

Régis Peytavi, Frédéric R. Raymond, Dominic Gagné, François J. Picard, Guangyao Jia, Jim Zoval, Marc Madou, Karel Boissinot, Maurice Boissinot, Luc Bissonnette, Marc Ouellette, and Michel G. Bergeron

Background: Current hybridization protocols on microarrays are slow and need skilled personnel. Microfluidics is an emerging science that enables the processing of minute volumes of liquids to perform chemical, biochemical, or enzymatic analyzes. The merging of microfluidics and microarray technologies constitutes an elegant solution that will automate and speed up microarray hybridization.

Methods: We developed a microfluidic flow cell consisting of a network of chambers and channels molded into a polydimethylsiloxane substrate. The substrate was aligned and reversibly bound to the microarray printed on a standard glass slide to form a functional microfluidic unit. The microfluidic units were placed on an engraved, disc-shaped support fixed on a rotational device. Centrifugal forces drove the sample and buffers directly onto the microarray surface.

Results: This microfluidic system increased the hybridization signal by ~10 fold compared with a passive system that made use of 10 times more sample. By means of a 15–min automated hybridization process, performed at room temperature, we demonstrated the discrimination of 4 clinically relevant Staphylococcus species that differ by as little as a single-nucleotide polymorphism. This process included hybridization, washing, rinsing, and drying steps and did not require any purification of target nucleic acids. This platform was sensitive enough to detect 10 PCR-amplified bacterial genomes.

Conclusion: This removable microfluidic system for performing microarray hybridization on glass slides is promising for molecular diagnostics and gene profiling.

© 2005 American Association for Clinical Chemistry

In recent years, DNA microarrays have become powerful tools for genomic research. Microarrays allow several thousands of captured nucleic acid probes to be spotted on a small surface on a solid support, generally a glass slide (1–4). Efforts have been undertaken to adapt the microarray technology for rapid identification of biomolecules by means of signal transduction after binding to specific probes attached to a solid support (5–10). Per se, there is a need for a rapid (less than 1 h) and sensitive microarray system suitable for the molecular diagnosis of infectious diseases, which involves the detection of a wide variety of microbial pathogens as well as associated virulence genes such as antimicrobial resistance genes or toxin genes (11, 12). Classic DNA microarray formats, such as Affymetrix’s Genechip or custom microarrays on glass slides, require hybridization times of ~18 h for detection of nucleic acids and are thus too slow for the rapid diagnosis of infectious diseases. Microfluidics is an emerging technology allowing the movement of minimum volumes in microscopic channels and chambers that are microfabricated in silicon, hard plastic, or soft elastomer polydimethylsiloxane (PDMS) (13). Fluid propulsion and controlled valves must be designed to allow sequential displacement of liquids into the desired channels and chambers (14). Microfluidic systems for nucleic acid hybridization with micropumps (15), pneumatic pumps (16), and syringe pumps (6) have been developed.
However, these systems are complex, expensive, and require special procedures for arraying bioprobes and for detecting hybridization signals. In this study, we built and tested a removable microfluidic structure allowing DNA hybridization on a glass slide microarray. After hybridization, microarrays can be analyzed externally with a standard scanner based on confocal microscopy for glass slide microarray analysis. We show that this microfluidic device allows the differentiation of single-nucleotide polymorphisms (SNPs) to identify *Staphylococcus* species in 15 min.

**Materials and Methods**

**CAPTURE PROBE HYBRIDIZATION**

All chemical reagents were obtained from Sigma-Aldrich and were used without further purification unless otherwise noted. Oligodeoxyribonucleotide capture probes, which were 5’-modified by the addition of a hexa(ethylene glycol) spacer and an amino linker, were synthesized by Biosearch Technologies. Four capture probes were used: (a), a *Staphylococcus aureus*-specific probe (5’-CGTATTATCAAAAGACGAAG-3’); (b), an *E. epidermidis*-specific probe (5’-CAIACGTGAAGTATACGTAT-3’); (c), an *S. haemolyticus*-specific probe (5’-CAAATTTAAGACGACGTATA-3’); and (d), an *S. saprophyticus*-specific probe (5’-AAAGCGGATTTACGTTTT-3’).

**FABRICATION OF THE ELASTOMERIC FLOW CELLS**

The microfluidic structures were fabricated using the PDMS replicating techniques reported by Duffy et al. (17). A wide variation of PDMS structures can be molded by use of microfabricated SU-8 micromolds (17–21). Two types of photoresist (i.e., SU-8 25 and SU-8 100) available from Microchem Inc. were used. SU-8 25 was used for the microchannel structures, and SU-8 100 was used for the reagent chambers. In the first step, SU-8 25 was processed on a 15.24-cm reclaimed Si wafer (Addison Engineering) to obtain the structures for the microchannels 25 µm in depth and the alignment marks for the second SU-8 25 layer. Subsequently, a thick layer (250 µm) of SU-8 100 was spin-coated over the substrate on which the molds for the microchannels had been created. This thicker layer was used to define the mold for the much larger reagent reservoirs. Because crosslinked SU-8 photoresists have lower optical transparency than their unexposed surroundings, the alignment marks can be observed readily, even when they are completely covered with a thick layer of the unexposed photoresist. In the pattern design, we compensated for possible alignment errors between the two layers of photoresist. The channels and chambers overlapped 50 µm in the connection areas to avoid possible disconnections caused by misalignment. Six identical molds were fabricated simultaneously on the 15.24-cm Si wafer for faster replication.

PDMS was purchased from Dow Corning. For polymerization molding of the flow cell, the base (Sylgard 184 silicone elastomer) and the curing agent were thoroughly mixed in a weight proportion of 10:1. Because of the thickness of the structures, low-temperature curing (i.e., 65 °C) in a convection oven was preferred over high-temperature baking. High temperature (e.g., 150 °C) causes substantial thermal stress at the interface between the SU-8 patterns and the Si substrate that can actually crack the substrate and peel off the SU-8 structures. Leveling of the PDMS on the substrate is required to achieve a uniform thickness over the entire flow cell.

**PREPARATION OF GLASS SLIDES**

The glass surface was functionalized based on the chemical reactions described by Joos et al. (22). All chemical reactions were carried out in polypropylene jars. Surfaces used were 25 × 75-mm microscope glass slides (VWR International). After sonication (Branson 1210 ultrasonic cleaner; Branson Ultrasonics Corporation) for 1 h in deionized water, the slides were sonicated for 1 h in 40 mL of 100 g/L NaOH, washed several times with deionized water, and dried under a stream of nitrogen. The slides were then sonicated in an aminopropyltrimethoxysilane solution (2 mL of water, 38 mL of methanol, and 2 mL of aminopropyltrimethoxysilane) for 1 h, washed with methanol, dried, and baked for 15 min at 110 °C. The amine-modified slides were activated by sonication overnight in 40 mL of 1,4-dioxane containing 0.32 g (2 mmol) of carbonyldimidazole as a coupling agent, washed with dioxane and diethyl ether, and dried under a stream of nitrogen.

**MICROARRAY PRODUCTION**

Microarrays were fabricated based on the method previously reported by Schena et al. (23). Oligonucleotide probes at 10 µmol/L in phosphate-buffered saline (pH 7.4; Sigma-Aldrich) supplemented with 1 mmol/L EDTA were diluted 2-fold by the addition of Array-it Microspoting Solution Plus (Telechem International). Capture probes were spotted in duplicate with a Virtek SDDC-2 arrayer (Bio-Rad Laboratories) equipped with SMP2 pins (Telechem International). Each spot had a volume of 0.6 nL and a diameter of 60–80 µm. Subsequently, the slides were dried overnight, washed by immersion in boiling 0.1% Igepal CA-630 for 5 min, rinsed in boiling ultrapure water for 5 min, and dried by centrifugation for 5 min under reduced pressure (SpeedVac plus from Thermo Savant). The slides were stored at room temperature in a dry and oxygen-free environment.

**PCR AMPLIFICATION AND AMPLICON LABELING**

Amplicons of 368 bp were generated either by standard PCR or by asymmetrical PCR amplification of purified staphylococcal genomic DNA using the *Staphylococcus* specific primers described previously (24). Genomic DNAs were purified from strains *S. aureus* ATCC 43300, *S. epidermidis* ATCC 14990, *S. haemolyticus* ATCC 29970, and *S. saprophyticus* ATCC 35552, as described previously (24). Fluorescent Cy dyes were incorporated during asymmet-
ric PCR amplification (25). Cy-labeled dUTP nucleotides (Amersham Biosciences) were used at a concentration of 0.02 mM in a 25-μL PCR mixture containing 0.02 mM dATP, 0.05 mM dCTP, 0.05 mM dGTP, 0.05 mM dTTP, 5 mM KCl, 1 mM Tris-HCl (pH 9), 0.1 mL/L Triton X-100, 2.5 mM MgCl₂, 0.5 U of Taq DNA polymerase (Promega), 0.2 μM primer TstaG765, primer TstagG422 at 0.2 μM for standard PCR or at 0.005 μM for asymmetric PCR, and 1 × 10⁴ to 1 ng of purified staphylococcal genomic DNA (equivalent of 10 to 1 × 10⁵ genome copies per PCR reaction). Thermal cycling for PCR amplification (180 s at 94 °C followed by 40 cycles of 30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C) was carried out on a PTC-200 DNA Engine thermocycler (MJ Research). For detection limit assays, PCR amplifications were performed using the equivalent of 1, 10, 100, 1000, and 10 000 genome copies purified from S. aureus strain ATCC 43300 to evaluate the minimum number of bacterial genome copies that can be detected by the microfluidic platform. Approximately 9 Cy-labeled nucleotides per amplicon were incorporated during PCR.

DNA MICROARRAY HYBRIDIZATION AND DATA ACQUISITION

We mixed 5 μL of amplified PCR reaction mixture containing the Cy-labeled PCR amplicons with 15 μL of hybridization buffer (8× SSPE (OmniPur; EM Sciences), 0.4 g/L polyvinylpyrrolidone and 400 mL/L formamide). Passive hybridization was performed in self-sticking, 20 μL, 15 × 13-mm Hybri-well hybridization chambers (Sigma-Aldrich). Amplicons produced by standard PCR were denatured by heating at 95 °C for 5 min. Hybridization buffer containing the labeled sample was introduced into the chambers, and hybridization was conducted for 5 min at room temperature. After hybridization, the microarrays were washed with 2× SSPE containing 1 g/L sodium dodecyl sulfate for 5 min at room temperature and rinsed once with 2× SSPE for 5 min. The microarrays were then dried by centrifugation at 1350 g for 3 min. The slides were then scanned using a ScanArray 4000XL (Packard Bioscience Biochip Technologies), and fluorescent signals were analyzed using its QuantArray™ software.

For flow-through hybridization, a unit consisting of a glass slide and our homemade flow cell was used. This unit was placed on a prototype plastic disc support, and the whole platform was fixed on the hub of a motor controlled by a computer (Fig. 1C). The labeled sample was prepared in the same way as for passive hybridization. Sample (2 μL) and 10 μL of washing and rinsing buffer were loaded on the microfluidic unit just before spinning the disc. The disc was spun at different speeds to sequentially burst the centrifugal valves and to allow the sample (12g), washing buffer (44g), and rinsing buffer (50g) to flow through the hybridization chamber. The disc was spun at high speed (100g) for 1 min to dry the slide and then scanned as described above for passive hybridization.

**Results**

**DESIGN OF THE HYBRIDIZATION UNITS**

This research is aimed at developing a simple, automated, and affordable DNA hybridization unit using centrifugal force for moving reagents. This system should be rapid, discriminative, and sensitive enough to be used for direct detection of microbes directly from clinical specimens. Amino-linker oligonucleotides specific to different staphylococcal species are used as capture probes. The capture
probes are immobilized on 4 linear arrays of 5 × 70-μm spots on a standard 75 × 25-mm glass slide. A self-contained hybridization process is performed in a flow cell designed for the compact disc (CD) platform. The flow cell consists of a hybridization column aligned with the DNA microarrays spotted on the glass slide, a sample chamber, a washing buffer chamber, and a rinsing buffer chamber. The reagent chambers are connected to the hybridization column with a 50-μm wide and 25-μm deep microchannel. The flow cell is aligned to adhere to the glass slide to form a nucleic acid hybridization unit. The microfluidic PDMS units and the glass slide with the capture probes array are pressed together, and the hydrophobicity of both materials allows leakage-free microfluidic channels. With this prototype, up to 5 microfluidic units can be mounted in the acrylic plastic CD platform machined by a computerized instrument (Fig. 1). The reagents are positioned to be displaced through the hybridization column by centrifugal force in a sequence beginning with chamber 2, up to chamber 4. Capillary valves contain the reagents in their specific reservoirs. The physical principle involved in capillary valves is based on the surface tension, which develops when the cross section of a hydrophilic capillary expands abruptly (26, 27). The flow sequence is achieved by shifting the balance between the capillary forces and centrifugal pressure (28). By varying the angular velocity of the driving motor, the flow rate of the different solutions can be controlled. The sample containing the labeled amplicons (chamber 2) is released first and flows over the 140-nL hybridization chamber (chamber 1), where the oligonucleotide capture probes are spotted on the glass support. The wash buffer (chamber 3) and the rinsing buffer (chamber 4) flow sequentially at higher angular velocities and are used to wash the nonspecifically bound targets after the hybridization step. After running through the hybridization chamber, waste liquids are collected in a furrow engraved in the CD plastic platform.

In the flow-through system, the total amount of targets transported to the spot from the bulk solution was approximated by diffusion layer theory (29). Under laminar flow conditions, when a sample solution flows over the capture arrays, the target DNA in a layer close to the spot surface will be depleted during the hybridization reaction. Close to the surface of the chamber, the velocity of the fluid approaches zero and can be regarded as forming a diffusion boundary layer having a thickness inversely proportional to the cubic root of the stream velocity (29). Within this layer, depletion to some degree is expected despite the mass transport caused by convection (30). The “diffusion layer thickness” can be estimated from Eq. 1. The equation is derived from a case of diffusion layer inside a circular tube (29) and has been adapted for laminar flow between two parallel plates of infinite width shown in Fig. 2:

\[
\sigma = \frac{1}{0.67} \left( \frac{DHx}{2v_0} \right)^{1/3}
\]  

(1)

where \( D \) is the diffusion coefficient of the target molecules, \( H \) represents the depth of the hybridization chamber, \( x \) is the distance from the edge of the measured region along flow direction, and \( v_0 \) is the maximum velocity of the fluid, which is the velocity at the center of the chamber. \( \delta \) represents the diffusion layer thickness. A parabolic velocity profile is expected in the vertical direction. Because the width (the transverse dimension, i.e., \( w_0 \) in Eq. 2) of the plate is substantially larger than the height, a flat velocity profile and, consequently, a uniform accumulation of targets are expected in width direction of the hybridization surface (400 × 3600 μm).

\[
F_{\text{flow}} = \frac{3w}{4v_0} c_0 V (2D^{2/3}x^2)^{1/3} H^{4/3} v_0^{-2/3}
\]  

(2)

where \( F_{\text{flow}} \) is the total molar concentration of target molecules on the hybridization surface, \( w_0 \) is the width of the hybridization chamber, \( w \) is the diameter of the spots, \( c_0 \) is the sample concentration, and \( V \) is the sample volume. The specification of the microfluidic design and operational conditions was guided by this equation. Note that if all the other variables remain constant, the depth of the hybridization chamber is the most sensitive factor affecting the accumulation of captured DNA. Smaller depths yield higher accumulation.

For passive hybridization, the accumulation of targets on the hybridization surface can be predicted by Eq. 3 (31):

\[
F_{\text{psv}} = 2c_{A0} \Delta x \sqrt{\frac{Dt}{\pi}}
\]  

(3)

where \( F_{\text{psv}} \) is the accumulation of the targets, \( c_{A0} \) is the concentration of target at the surface, and \( t \) is the time.
FLOW-THROUGH HYBRIDIZATION IN 15 MIN

The flow-through hybridization unit combines a PDMS flow cell juxtaposed with a glass slide a few minutes before the experiment without performing any surface treatment or adhesion step. This microfluidic system allows robust control of valve opening to sequentially release the contents of different chambers. Indeed, Fig. 3 shows that the bursting range in rotation per min for each of the 3 centrifugal valves of our CD microfluidic system do not overlap.

Each staphylococcal amplicon at 10 nmol/L generated by asymmetric PCR was hybridized by both passive and flow-through hybridizations. For flow-through hybridization, loading of the reagents was performed immediately before spinning the disc platform to avoid reagent evaporation. A spin speed was selected to obtain a sample flow rate of 0.4 μL/min in the hybridization chamber, which corresponds to a hybridization time of 5 min considering that 2 μL of sample was loaded on the microfluidic unit. This hybridization time is identical to the time used in the passive hybridization experiments. After the hybridization step, the spin speed of the platform was increased to sequentially burst the centrifugation valves, releasing 10 μL of washing buffer and 10 μL of rinsing buffer, respectively, into the hybridization chamber. These two buffers flowed through the hybridization chamber with a mean flow rate of 2.2 μL/min in ~9 min. Finally, there was a 1-min drying step (high spin speed). The total time for the entire hybridization process was therefore ~15 min. Subsequently, the PDMS microfluidic flow cells were peeled off, and the hybridized microarrays were scanned. Plotting of the fluorescence intensity revealed that flow-through hybridization in a 140-nL chamber was more sensitive than passive hybridization in the 20-μL chamber. Five min of hybridization with 10 nmol/L of amplicon generated from S. aureus, S. epidermidis, S. haemolyticus, and S. saprophyticus showed ratios of 9.5, 13.5, 18.7, and 6.9, respectively, between flow-through and passive hybridizations (Fig. 4). Hybridizations of amplicons generated by standard PCR amplification from the equivalent of 1, 10, 100, 1000, or 10 000 genome copies were performed with the flow-through hybridization system. We found that the equivalent of as little as 10 genome copies of starting material was sufficient to produce an unambiguous hybridization signal (Fig. 5).

DIFFERENTIATION OF 4 CLINICALLY IMPORTANT Staphylococcus species

Staphylococcus-specific PCR primers targeting the tuf gene were used to amplify a 368-bp fragment from S. aureus, S. epidermidis, S. haemolyticus, and S. saprophyticus purified genomic DNAs. Species-specific capture probes targeting these 4 staphylococcal species were arrayed on glass slides and hybridized with the 4 different staphylococcal amplicons. The results demonstrated that it was possible to detect and differentiate the 4 different staphylococcal tuf amplicons (Fig. 6). The S. epidermidis-specific oligonucleotide probe was designed in a specific area of its genome that differs from the S. aureus sequence by only a SNP. A nucleotide analog was added at a strategic location in the S. epidermidis probe to make it more discriminative for the S. aureus amplicon. With this strategy, hybridization of S. aureus amplicons gave a fluorescence signal ~6 times stronger with the S. aureus probe compared with the S. epidermidis probe (Fig. 6). The other oligonucleotide capture probes had at least 3 nucleotide mismatches with the nonhomologous Staphylococcus amplicons, and no substantial cross-hybridization was observed. In addition, a mixture of the 4 Staphylococcus amplicons (each at 1 nmol/L), hybridized on the same glass slide, was analyzed to test the specificity of the capture probes. The results demonstrated that the hybridization system was able to differentiate the 4 staphylococcal species with high specificity and sensitivity.

Fig. 3. Bursting range for each of the 3 centrifugal valves of the CD microfluidic system.

The bursting range in RPM for each valve was determined by performing flow-through hybridizations using 15 independent microfluidic units.

Fig. 4. Comparison between the sensitivity of labeled staphylococcal amplicon detection in passive hybridization vs flow-through hybridization.

Both passive and flow-through hybridizations were carried out for 5 min at room temperature with 10 nmol/L of 368-bp staphylococcal Cy-labeled amplicons. The graph shows the mean fluorescence intensity in units for each species-specific capture probe. Standard deviations (error bars) are for the results of 5 hybridizations.
microarray by our microfluidic system, showed fluorescence hybridization signals with intensities similar to those shown in Fig. 6 for each of the 4 species-specific capture probes.

Discussion

In recent years, microarrays have become tools of choice for gene expression profiling. The expression of thousands of genes can be monitored in a single experiment with this technology. Several investigators have attempted to adapt this technology to rapidly detect infectious agents in clinical specimens for diagnostic purposes (6, 8, 32–34). However, such systems are still in their infancy, and most of them require technologically complex systems with integrated heating/cooling (6, 8, 16).

This study reports the merging of standard microarray glass slide technology with a simple, low-cost microfluidic device. We demonstrated that nanoliter volumes of liquid can be moved precisely into channels and chambers on the glass slide surface created with a microfluidic elastomeric flow cell juxtaposed above the slide. This custom microarray hybridization microfluidic platform is easy to use, automated, and rapid. It uses standard glass slides compatible with commercial arrayers and scanners found in most academic departments. In this removable microfluidic system, the hybridization chamber is composed of a microfluidic network engrafted on a disposable, low-cost elastomeric material. This elastomeric material reversibly sticks without any adhesive or chemical reaction to the glass slide, forming together the microfluidic unit. Placed on a plastic CD-like support, the microfluidic units are spun at different speeds to control fluid movements. To simplify hybridization experiments using this device, buffer compositions and capture probe sequences are optimized to be compatible with room temperature hybridizations to avoid the need for a heat-

Fig. 5. Detection limit assays using the prototype microfluidic system. Hybridization to microarrays of an S. aureus-specific capture probe was performed using Cy-labeled tuf gene amplicons generated by standard PCR amplification of 1, 10, 100, 1000, or 10,000 genome copies of S. aureus. The graph shows the mean fluorescence intensity for each bacterial genome copy number tested. Standard deviations (error bars) are for the results of 4 hybridizations.

Fig. 6. Microarray hybridization applying the prototype microfluidic system. Hybridization to microarrays of species-specific capture probes targeting staphylococcal tuf sequences were performed with Cy-labeled tuf gene amplicons generated by asymmetric PCR amplification of 1 ng of genomic DNA purified from 4 staphylococcal species. (A), crude images of microarrays, hybridized 5 min at room temperature, with the 4 different staphylococcal labeled amplicons. (B), graphs showing the mean fluorescence intensity in units for each capture probe. Standard deviations (error bars) are for the results of 5 hybridizations.
ing device. Furthermore, this microfluidic system allows a drastic reduction in the volume of reagents needed for microarray hybridizations and does not require a PCR amplicon purification step, which may be time-consuming.

Among various pumping methods attempted in research of flow-through DNA chips, one is electroosmotic pumping (EOP) (35–40). However, DNA has a high negative electrophoretic mobility because of the large number of negative charges carried by its phosphate groups at most pH values. Consequently, EOP of DNA requires a high electroosmotic mobility buffer to overcome the negative electrophoretic mobility of the DNA molecules. Furthermore, DNA hybridization buffers often contain high concentrations of salt that reduce the electroosmosis effect and make the EOP a less effective approach (16). Pumping with mechanical pressure presents some advantages over the EOP approach as it is insensitive to pH, to the charge of moving molecules, and to salt concentration. However, high back pressures can be generated because of the high flow resistance caused by the small dimensions of the microchannels. Consequently, leakage is often a problem if the unit is not sealed. With the CD platform, the reagents are delivered by centrifugal force generated over the entire length of the liquid element. Therefore, local high pressure is avoided, and as a result, interface sealing is readily accomplished. In fact, no leakage was observed with this approach. In the present study, PDMS was selected to make the microchambers and channels. PDMS is a low-cost material that can be easily prototyped and can make reversible and watertight seals with glass slides. Each microfluidic unit, composed of the molded PDMS juxtaposed on a glass slide, was placed in a custom-made plastic disc support (Fig. 1). Centripetal force was used to move the liquid into the microfluidic chambers and channels as described previously by us (41, 42). The rapidity of the hybridization reactions prevents reagent evaporation problems that may be encountered in slower standard microarray hybridization methods.

In a passive hybridization system, a hybridization event requiring collision between a capture probe and the analyte relies solely on diffusion. In such systems, sensitivity is increased by means of long hybridization periods (43, 44). One advantage of flow-through hybridization is that, because of the shallow architecture of the microchannels, the probability of collision between the probe and the analyte is increased by the much shorter diffusion distance. This allows fluid movement of nanoliter volumes over the capture probes, thereby accelerating the hybridization kinetics (44–46). Using a microfluidic system, Chung et al. (47) showed a 6-fold increase in hybridization efficiency with the flow-through hybridization performed in a 33-μL chamber compared with the passive hybridization performed in a chamber of the same volume. In our work, we increased even further the kinetics of hybridization with a much smaller hybridization chamber (140 nL) combined with flow-through hybridization. Flow-through hybridization in such a small hybridization chamber allowed a substantial reduction in the reagent and sample volumes (1/10) while reducing hybridization time to 5 min. This rapid hybridization by means of our microfluidic system increased the kinetics of hybridization by an average of 10.5-fold compared with passive hybridization using 368-bp PCR amplicons as nucleic acid targets. By contrast, 30 min was required for hybridization in the flow-through hybridization system described by Chung et al. (47). Furthermore, there is no need to purify the target PCR amplicons before hybridization, thereby reducing the overall time of the assay.

To be useful for the diagnosis of infectious diseases in clinical laboratories, a molecular test should be highly sensitive, specific, and ideally, rapid and inexpensive. Our system showed a detection limit of 500 amol of amplified target. This detection limit is comparable to those obtained with microfluidic devices that are more complex to manufacture (mainly irreversibly bounded plastic devices actuated with active valves) and requiring much longer hybridization times (6, 48). One system using chemiluminescence has a detection limit of 250 amol, but requires a 3-h hybridization period (49), too long for practical use in clinical diagnostics (50). Here we report a microfluidic system that allows detection of PCR amplicons generated from the amplification of 10 bacterial genome copies, which is at least 1000 times more sensitive than results obtained by other groups using microarray hybridization on microfluidic devices (15). The addition of an amplicon concentration step, as well as the use of a microfluidic device for faster PCR thermocycling, should allow detection of even lower copy numbers.

In terms of specificity, this simple system differentiated 4 different Staphylococcus species with a post-PCR hybridization protocol of only 15 min. An artificial mismatch created by the addition of an inosine was incorporated in the S. epidermidis capture probe to further destabilize binding events with the S. aureus amplicons differing by only SNP. The nucleotide analog was located 4 nucleotides upstream from the SNP present in the S. aureus genomic DNA sequence. Our actual device design can accommodate up to 150 spots in the 140-nL hybridization chamber. This should be sufficient for some practical applications in the diagnostic field, but future improvements in spot density and hybridization chamber geometry will substantially increase the number of spots in the microfluidic hybridization chamber.

In conclusion, this microfluidic microarray system could become a tool of choice for the rapid identification of nucleic acid sequences in fields such as molecular diagnostics, detection of bioterrorism agents, food quality survey, and forensic analysis. This prototype paves the way toward the development of micro-total analysis system (μ-TAS) on a CD support, where sample preparation, PCR amplification, and microarray hybridization are performed in a 33-μL chamber of the same volume. This allows fluid movement of nanoliter volumes over the capture probes, thereby accelerating the hybridization kinetics (44–46). Using a microfluidic system, Chung et al. (47) showed a 6-fold increase in hybridization efficiency with the flow-through hybridization performed in a 33-μL chamber compared with the passive hybridization performed in a chamber of the same volume. In our work, we increased even further the kinetics of hybridization with a much smaller hybridization chamber.
and detection could be accomplished with a single portable device for point-of-care diagnostic applications.

We thank Yves Lechasseur for technical help in setting up the rotational platform, Eric Martel for mathematical assistance, and Guy Boissinot for work on the plastic support. This research project was supported by grants from “Valorisation de la Recherche duQuébec”, from “GénomeQuébec” and Genome Canada, and from the Canadian Institutes of Health Research (PA-15586). M.O. is a Burroughs Welcome Fund scholar in molecular parasitology and the holder of a Canada Research Chair in antimicrobial resistance.

References


