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Forensic DNA Analysis on Microfluidic Devices: A Review

ABSTRACT: The advent of microfluidic technology for genetic analysis has begun to impact forensic science. Recent advances in microfluidic separation of short-tandem-repeat (STR) fragments has provided unprecedented potential for improving speed and efficiency of DNA typing. In addition, the analytical processes associated with sample preparation—which include cell sorting, DNA extraction, DNA quantitation, and DNA amplification—can all be integrated with the STR separation in a seamless manner. The current state of these microfluidic methods as well as their advantages and potential shortcomings are detailed. Recent advances in microfluidic device technology, as they pertain to forensic DNA typing, are discussed with a focus on the forensic community.

KEYWORDS: forensic science, DNA typing, microfluidics, microchip technology

In the early 1990s, microfabrication technology acquired from the rapidly evolving electronics industry gave birth to a new era in analytical chemistry. These established techniques have been utilized to develop bioanalytical microfluidic devices, termed "lab-on-a-chip" (LOC) or micro-total analysis systems (μTAS) for their potential to incorporate many macroscale techniques onto a single microchip. Microdevices can be fabricated from a variety of substrates, most commonly silicon, borofloat silica, and polymeric substrates such as polydimethylsiloxane (PDMS), polycarbonate or poly(methyl methacrylate; PMMA) (1). Microchip dimensions are typically on the order of 1–10 cm in length and width, and 1–2 mm in height. Substructures are built into the devices for fluidic manipulation using wet chemical (hydrofluoric acid) etching (borofloat silica), reactive ion etching (silicon), or hot embossing (polymeric devices). Initially, analytical microchips were utilized solely for separations that were typically performed on capillary, providing proof-of-concept for the technology transfer from the capillary to the microchip platform (2). Despite the realization that the potential for microchip technology lay far beyond the equivalent processes that could be performed on a capillary, the development and integration of multi-process functionality into the very devices used for separation (as inferred by the name "lab-on-a-chip") lagged substantially behind advances in chip-based separations. However, the last half decade has seen a burst of research activity in chip-based sample preparation, yielding, as delineated in the following subsections, methods for cell sorting, DNA extraction, DNA quantitation, PCR amplification, and DNA separations as processes that can be effectively performed on microdevices (3,4). As such, microdevices have the potential to revolutionize forensic DNA testing with state-of-the-art analytical technology.

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Historically, forensic DNA analysis has utilized slab gel electrophoresis for separation of restriction fragment length polymorphism (RFLP) fragments and polymerase chain reaction (PCR) products (5). The forensic DNA community saw a dramatic change upon the introduction of capillary electrophoresis (CE), and since the mid-1990s, separations of PCR products have routinely been performed using CE in forensic laboratories (6,7). CE brought about a limited degree of automation of separation and detection, unavailable in the slab gel format. Separations that typically required several hours on the slab gels could be completed in approximately 30 min (8). For clinical DNA analysis, the advent of microchip electrophoresis has reduced the separation time by yet another order of magnitude [e.g., (9)]. It is not unreasonable to envision similar improvements for forensic analyses. For the mere advantage in faster DNA separations alone, microchips are clearly worth implementation into the forensic DNA community; however, with CE and microchip electrophoresis, the overall processing time per sample has not been significantly reduced. Therein lies the greatest advantage of the analytical microchip—the potential to incorporate sample processing steps (DNA extraction, quantitation, amplification, and separation) onto microdevices for fast, automated processing. Whether the ultimate embodiment of the device is in the form of modules or a totally integrated system will be driven by the needs of the forensic science community.

The analytical microchip has numerous advantages over the current technology, including increased efficiency, decreased sample handling, and decreased reagent and sample consumption. In the forensics community, decreased sample handling is a key feature, as there is less opportunity for sample contamination during the processing steps. The use of microdevices has resulted in increased efficiency of numerous methods due to the dramatic decrease in reaction volumes and high surface-area-to-volume ratio when compared with the conventional processes. The volumes typically involved in fluid manipulation on microdevices are in the nanoliter range, rather than the microliters associated with conventional methods. The resulting benefit would be a significant reduction in reagent consumption, leading to lower analysis costs. These features make analytical microchips the ideal new technology platform for forensic DNA analysis.

Motivation

With the backlog of DNA evidence to be analyzed in our nation's forensic laboratories, the need for a new era in forensic DNA analysis technology is increasingly evident. According to the National Institute of Justice 2004 Annual Report, estimates place the DNA backlog at approximately 800,000 convicted offender samples and approximately 485,000 criminal cases awaiting analysis in our nation's state and federal crime labs, primarily due to limited resources, such as time and funding (10). The backlog undermines the full potential of DNA databases, as numerous samples have not been typed and are, therefore, not yet included. Currently, forensic DNA analysis, including DNA extraction, quantitation, amplification, and separation, requires approximately 12–36 h of linear time to complete, much of which requires direct technician time. An integrated forensic DNA analysis microdevice, however, could complete these processes on the order of 1–2 h—all in an automated system, reducing not only analysis time but also user intervention. It is the tremendous inherent potential of microdevices that stands to revolutionize forensic DNA analysis and warrants attention.

Sample Processing Steps

As with almost any form of genomic interrogation, forensic DNA analysis requires numerous sample preparation steps prior to the analytical step that generates an STR profile, all of which could benefit by translation to the microchip format. The steps discussed in detail in the following section include differential extraction, DNA purification, DNA quantitation, PCR, and short tandem repeat (STR) fragment separations. While the theory behind each processing step remains largely unchanged, the specific methods have been altered to varying extents, to accommodate the microchip format and improve performance. Although not an exhaustive discussion of the pertinent literature, the following sections present the current state of microfluidic technology as applied to these analytical processes by alluding to select reports in the literature that we feel are particularly relevant.

Cell Sorting/Differential Extraction

Analysis of sexual assault evidence requires the use of "differential extraction" to obtain separate fractions of male and female DNA. Differential extraction is one bottleneck in the automation of forensic DNA analysis. While significant improvements to the other "core" processes have been addressed to enhance throughput, differential extraction has advanced relatively little in the same period. The conventional method involves differential lysis of sperm and epithelial cells, employing centrifugation to separate the epithelial cell DNA (female fraction) from the sperm cells (male fraction). Although not routine, microfluidic control of flow on microdevices has been shown possible using centrifugation as a driving force in a compact disc-like device (11) and for clinical applications that include preparation of plasma from whole blood (12). While the approach has not been applied to forensic differential extraction, other methods have been pursued as an alternative for obtaining male and female fractions on microfluidic devices.

One microscale method for differential extraction (13) used low-power sonication for selective epithelial cell lysis and a filter (pore size not indicated) to separate the epithelial cell lysate from the intact sperm cells/heads. Sperm were subsequently lysed using more-stringent conditions, followed by DNA extraction from both cell lysates on the same device (Fig. 1). The differential extraction process using this microfluidic-based system was fully automated and yielded male and female fractions of DNA in <3 h. Results indicating the purity of the recovered male and female fractions have not yet been reported in the literature to our knowledge. The work is the only known example of true microchip-based differential extraction, although one could envision slight alterations to the conventional method to obtain equivalent results, such as sorting the cell types and extracting the DNA from each fraction independently.

Numerous approaches for cell sorting have been demonstrated on microdevices, with only one specifically addressing the separation of sperm and epithelial cells. In a very simplistic approach, our laboratory demonstrated the separation of sperm and epithelial cells on a microdevice containing a single straight microchannel

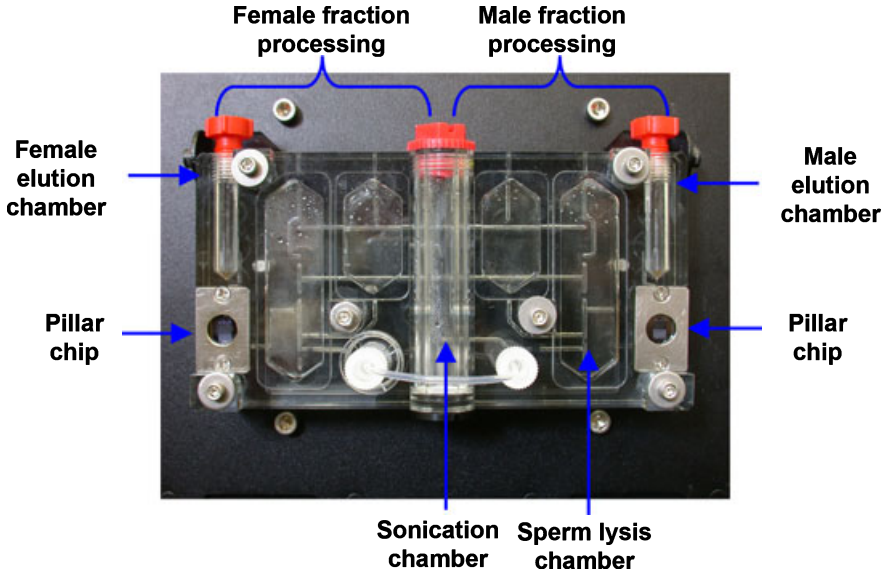

FIG. 1—*Sample processing cartridge developed by Microfluidic Systems, Inc. for forensic differential extraction in under 3 hours. The device utilizes fluidic pumps, solenoid valves, rotary valves, and ultrasonic horns interfaced to the device through a pneumatic manifold to control fluidic movement. In an automated fashion, the biological material is eluted from the swab, epithelial cells selectively lysed using sonication, the intact sperm cells separated from the epithelial cell lysate using a filter, and the DNA purified from each using a silicon solid phase. (Photo courtesy of Microfluidic Systems, Inc.)*

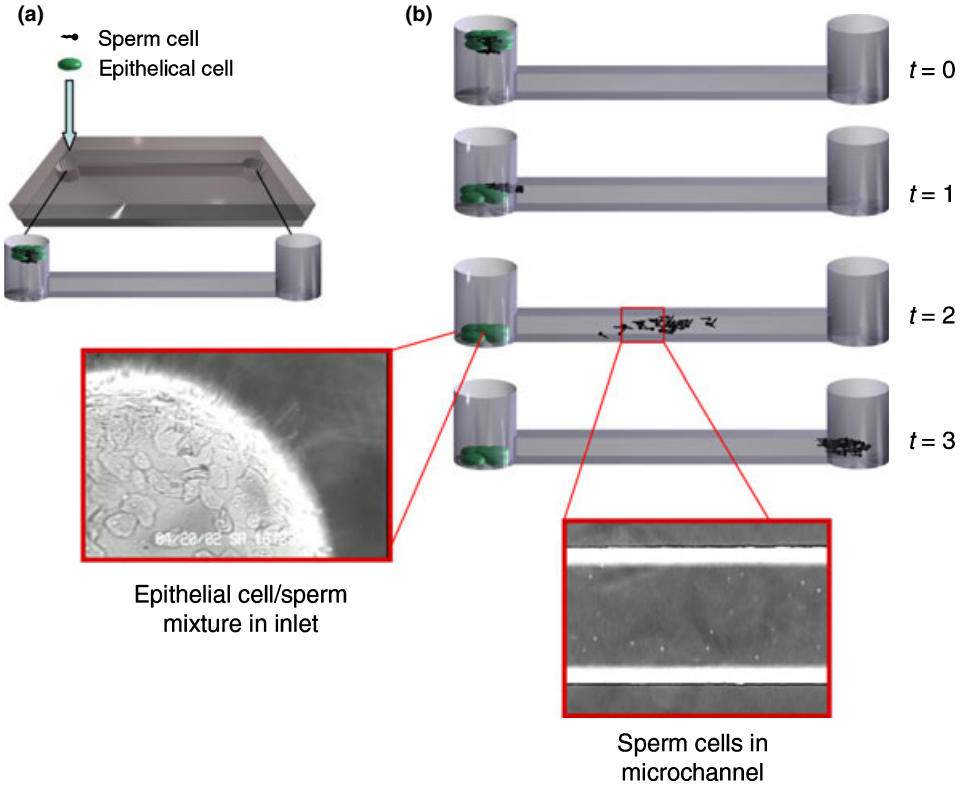

FIG. 2—*Separation of sperm and epithelial cells on a microfluidic device. (a) The sperm and epithelial cell mixture is added to the inlet reservoir of the microdevice. (b) The epithelial cells sediment to the bottom of the inlet reservoir (t = 0). After 5 min (t = 1), flow is induced (t = 2) to mobilize the sperm cells, whereas the epithelial cells are retained in the inlet reservoir, adsorbed to the glass substrate. At t = 3, the sperm cells are collected in the outlet reservoir, from which further sample processing (DNA extraction, PCR amplification, and DNA separation) can occur. Photomicrographs of the inlet reservoir and channel show the epithelial cells and sperm cells, respectively, at a late stage of the separation. (Figure adapted from and reproduced with permission from Horsman et al., Analytical Chemistry 2005, 77, 742–9. Copyright 2005 American Chemical Society.)*

(14). The separation was based upon differential sedimentation rates of the two cell types possessing distinct physicochemical properties. As shown in Fig. 2, a mixture of sperm and epithelial cells are introduced to the inlet reservoir. After 5 min of settling time, the sperm cells were mobilized in the channel with a negative-pressure, syringe-pump driven flow. The sperm cells were then collected in the outlet (right) reservoir, while the epithelial cells remain in the inlet (left) reservoir. A shortcoming of the approach is the potential presence of free DNA in the sample, most likely from lysed epithelial cells, although this can be addressed through modification of the microchannel surface. While the method is the only published microscale technique specifically addressing sexual assault evidence, other established macroscale cell-sorting techniques are potentially applicable to this sample.

A common cell sorting mechanism that has gained widespread use in the macroscale is fluorescence-activated cell sorting (FACS). The Quake lab (15,16) demonstrated FACS on the microscale, in silicon/PDMS microdevices. The technique required that one (or both) of the cell types be fluorescently tagged for detection by the system and, as a result, lead to a reproducible method of cell sorting. However, a major drawback was the need to fluorescently tag one cell type and utilize an optical setup or fluorescence detection, which, for a stand-alone instrument, would add substantially to the cost of the microchip method. Schoell et al. (17,18) applied flow cytometry, in the macroscale, to the separation of sperm and epithelial cells, using fluorescently tagged antibodies to cell surface antigens or a DNA stain to distinguish the cell types. In the case of the DNA stain, the cells were distinguished via stain intensity and, thus, ploidy. The results presented were notable as they yielded higher sensitivity than the conventional differential extraction technique. However, a drawback to the use of antibodies is that the extent of degradation of cell membranes in forensic samples is unknown and could substantially diminish the antibody recognition of cell type. In addition, the sensitivity of the technique using the DNA stain is unclear when based solely on ploidy. The authors indicated that use of the technique would require changing the method of evidence collection from a vaginal swab to flushing the vagina with an isotonic solution.

Sperm capture was also demonstrated using dielectrophoresis, in which cells differentially migrate toward a concentrated electric field depending upon their physical properties, on a microdevice (19). The method, developed for the infertility industry, has obvious extension to forensic differential extraction, as the electric field can be utilized to trap the cells of interest, including sperm cells in a cellular/biological mixture, based upon their physicochemical properties such as conductivity and permittivity. A potential drawback to the technique is the adherence of sperm cells to the microchip substrate at the trapping site. An analogous technique using an acoustic field to trap (and levitate) the sperm cells in the middle of the microchannel (rather than at the microchannel surface) is being developed to circumvent the limitation (Fig. 3). The particle-trapping technique utilizing acoustic standing waves has been developed by the Laurell and Nilsson groups (20,21) and was recently applied to forensic differential extraction (22). Upon infusion of the sample, sperm cells were trapped in the near field of an ultrasonic resonator above a piezoceramic microtransducer, whereas free DNA (from lysed epithelial cells) was not retained but directed to a reservoir for subsequent testing. After washing the trapped

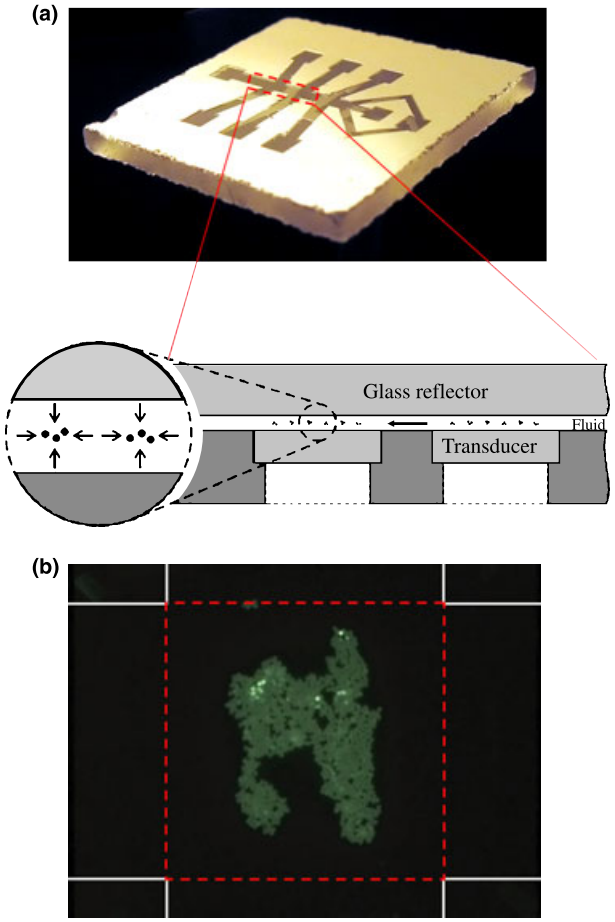

FIG. 3—*Acoustic trapping of cells in a microfluidic device. (a) Photograph of the glass channel structure of the microdevice. The microtransducers (seen in cut-away view) are placed directly below the intersections of the channels. A cut-away view of the center of the main channel in the acoustic trapping device is also shown. Cells flow through the microfluidic channels and directly over the microtransducers (fabricated into a printed circuit board layer). Upon activation of the transducers, an acoustic standing wave is set up in the microdevice, resulting in a pressure minimum in the center of the channel (as indicated), where cells are trapped. (b) Photomicrograph illustrating the trapping of fluorescently tagged polystyrene beads in the microdevice. The beads are trapped at the intersection of the main channel and a side channel. The channel walls are indicated in white. Outlined in red is the approximate size of the transducer, above which the cells are trapped. (A portion of the figure (a, bottom) adapted with permission from Nilsson et al., Acoustic Trapping of Cells in a Microfluidic Format. Micro Total Analysis Systems 2005, Proceedings of the mTAS 2005 Symposium, 9th, Boston, MA. 2005;5. Photographs courtesy of T. Laurell and J. Nilsson of Lund University, Sweden.)*

cells with buffer, the sperm cells were released and directed into a second reservoir.

A macroscale cell sorting method was also developed using an 8 μm filter, which allows sperm cells to pass through the filter, while epithelial cells were retained (23). A drawback to the method is that any free DNA or nuclei from lysed epithelial cells will penetrate the membrane and, thus, contaminate the sperm cell fraction. In an alternate manifestation (24), a 2 μm filter was utilized to separate epithelial cell lysate from sperm cells, which circumvents the problem encountered above with respect to lysed epithelial cell DNA contaminating the sperm cell fraction. As clogging can be a problem with filter-based separation mechanisms, the authors included an 11 μm prefilter. While filter-based methods are not currently performed on microdevices, implementation of the separation on a microchip would be relatively straightforward, with the major hurdles likely being the microfabrication and potential clogging of the microchannel.

Although not specifically developed for the microchip platform, Eisenberg (25) reported an antibody-based approach to capture sperm cells. A cocktail of antibodies to sperm cell surface antigens was anchored to magnetic beads over which the sample flows. The beauty of the approach is the ability to exploit the specificity of Ab–Ag binding to selectively capture sperm cells from evidentiary material that may be comprised of a mixture of sperm cells, white blood cells, epithelial cells, cell lysates, etc. The major drawback, as discussed with the FACS method above, is the unknown level of sperm cell membrane degradation that will render the sperm cell unamenable to antibody complexation. Additionally, the introduction of unlysed epithelial cells into the column could significantly compromise the efficiency of the separation. Although the method has not been demonstrated on a microfluidic platform, one could envisage the translation of the separation mechanism to a microdevice, with the provision that the drawbacks described could be effectively addressed.

As indicated, the translation of conventional differential extraction to microdevices is still in its infancy. While only a few microchip methods specifically addressing the separation of sperm and epithelial cells/DNA have been demonstrated to date, a number of methods have shown potential for application to the particular analysis. These microchip-based methods, once fully developed, will require rigorous validation and testing with casework samples to fully demonstrate their capabilities and benefits. Integration of differential extraction with downstream sample processing techniques also remains to be demonstrated.

DNA Purification

Historically, conventional methods utilized to purify DNA for forensic analysis have typically included an organic extraction (e.g., phenol chloroform) step. These protocols, although effective for recovering high-molecular-weight DNA for downstream analysis, require multiple vortexing, centrifugation, and transfer steps that are not easily translated to microfluidic platforms. More recently developed techniques, however, have shifted to the use of solid phase extraction (SPE) methods (Qiagen, DNA IQ, etc.), reducing the time required for the extraction while maintaining recovery and sample purity and integrity. In addition to enabling faster sample preparation, these protocols are more easily translated into microdevice formats. Solid phases such as silica beads, sol-gels, or ion exchange resins can be easily packed into microdevices to create a SPE bed or column for DNA purification. Additionally, a variety of novel solid phases can be created in microdevices during the fabrication process that are suitable for sample purification. Although many of these methods were developed for clinical diagnostic applications, the devices and techniques should be readily translatable to forensic applications, and a number research groups are focusing efforts on concerns unique to the forensic community. The advancement of these methods is key to the establishment of sample preparatory steps in microdevices, either as stand-alone systems or as an essential component of a fully integrated, microchip-based analysis to speed casework handling.

The first instance of a truly microchip-based SPE was published by Christel et al. (26) in 1999. A silicon microdevice containing pillars for high surface-area-to-volume ratios to increase DNA adsorption was designed by the group for the purification and concentration of DNA for PCR amplification. As commonly utilized with other silica-based SPE methods, a chaotropic salt (guanidine

HCl) was used as the binding/load solution, followed by an ethanol wash to remove proteins, and DNA elution with water. This represented the first microchip-based DNA purification accomplished; however, the complex reactive-ion-etching protocol utilized to fabricate the solid phase, combined with the high cost to produce such a device, limits the potential utility of the format. In addition, capture efficiencies reported with the device using stock lambda DNA were only 50%, limiting its utility for low copy purifications where retention of DNA is imperative. Cady et al. (27) have employed a similar design concept with the goal of providing a device with higher binding capacity (>200 ng). However, it was not possible to gauge the effectiveness of the device for forensic analysis as no extraction efficiencies were reported. While efforts toward extracting DNA from larger volume samples are certainly needed, extraction must yield a reasonably concentrated DNA solution amenable to downstream processes (e.g., PCR). With DNA eluted from the solid phase in an unusually large volume (250 μL) in the work (27), the likelihood that such a method could be effectively integrated with other microchip processes is minimized. While the extraction efficiencies attainable with chromatographic solid phases created by microfabricated pillars are not readily apparent in the literature, there is no question that, in the future, high-surface-area, functionalized solid phases may prove to be a robust and efficient means for DNA purification. As depicted in Fig. 4, functionalized surfaces containing pillars and/or pores that provide increased surface areas, and a concomitant increase in capacity, for DNA binding can be microfabricated, without the issues of back pressure and device filling associated with packed solid phases. These new phases may provide reproducibly uniform surfaces with which to extract DNA.

In a more direct translation of current macroscale extraction protocols, other microchip-based purification systems have focused on utilizing a silica bead or silica sol-gel matrix solid phase for DNA purification. The use of these solid phases for DNA extraction was first miniaturized in a capillary format to demonstrate the utility of the proposed method in the microscale (28). Tian et al. (28), employing a 500 nL capillary-based chamber packed with a silica particles, established that PCR-amplifiable DNA (with 80–90% of proteins removed during the load and wash steps) could be obtained from white blood cells with high extraction efficiencies (70%). The work demonstrated both the suitability of microminiaturized SPE methods for a wide variety of biological species (white blood cells, cultured cells, whole blood) and the feasibility of incorporating such methods into microfabricated devices.

The microscale extraction technique was extrapolated to silica microdevices by Wolfe et al. (29) who evaluated a variety of silica and silica/sol-gel matrices for DNA extraction. In the work, the authors highlight the potential problem associated with using silica beads or particles in a microdevice: the tendency of these particles to pack more tightly under flow as multiple extractions proceed, thus affecting the reproducibility of repeated extractions. The challenge was circumvented in designs such as those proposed by Christel (26) and Cady (27), which contain microfabricated pillars that are part of the channel. Recent work in our lab suggests that if the devices are single use, packed silica bead solid phases are acceptable purification phases for DNA extraction. Alternatively, sol-gels, liquid colloidal suspensions of silica-based materials that can be acid or base catalyzed to gel in place, have been demonstrated as efficient, reusable solid phases as described by Wolfe et al. (29). These solutions are simply flowed into microchambers as liquids and allowed to gel to form a porous DNA extraction bed. The catalyzed reaction can be controlled to create pores that allow enough surface area for the binding of DNA, as demonstrated by Wu et al. (30), who utilized the phase to extract DNA from bacterial (anthrax), viral (varicella zoster and herpes simplex), and human (blood) sources, with >65% extraction efficiency from blood. In a later translation of the work, Wen et al. (31) utilized a photopolymerizable sol-gel monolith to extract DNA in a capillary-based system, which was further modified for microchip-based extraction (32). The photopolymerization step allows for easy and precise formation of the solid phase within the microdevice, without the use of retaining weirs or other microfabricated features. In addition, the solid phase has recently been incorporated into a novel two-stage microdevice that was developed for DNA extraction from blood—a C18 reverse phase column for protein capture (stage 1) in series with a monolithic column for DNA extraction (stage 2) (32). The device had a high capacity for DNA in blood (>240 ng) and was found to achieve ~70% extraction efficiency. Further, the sol-gel extraction medium can be not only be used alone, but also as a glue, to immobilize a silica bead phase, maintaining a reproducible extraction column from run to run. The latter solid phase was evaluated by Breadmore et al. (33), who optimized flow rates and loading pH to affect a sample purification in 15 min from bacterial sources (anthrax and salmonella) and whole blood. In addition, intra- and interdevice reproducibility was demonstrated, with as high as 79% extraction efficiency achieved.

The utility of this solid phase and extraction protocol was also demonstrated for DNA purification from sperm cells, with a view

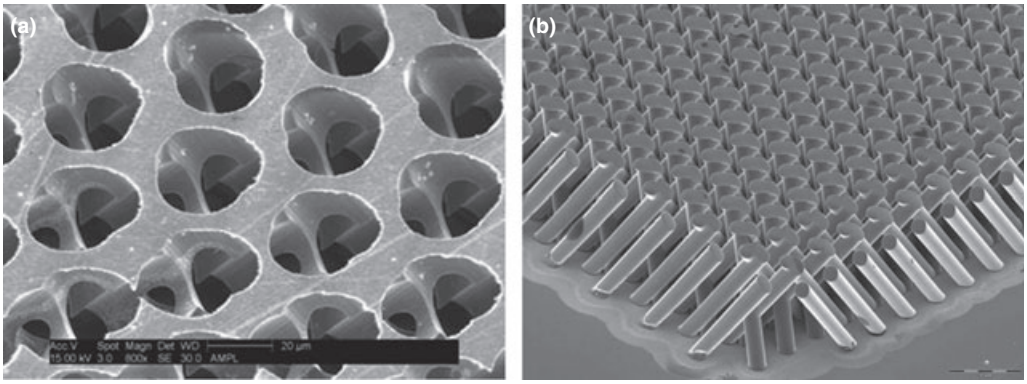

FIG. 4—*An example of a high surface area structure microfabricated out of plastic (polymethylmethacrylate). These structures can be functionalized for efficient capture of nucleic acids, providing large surface areas and increased binding capacity for DNA extractions, as well as reproducible and low-back-pressure columns for purification. (a) A channeled structure where the individual channels are 30 μM in diameter. (b) Complementary (inverse) structure to that shown in (a). (Photo courtesy of HT Microanalytical, Inc. [Albuquerque, NM].)*

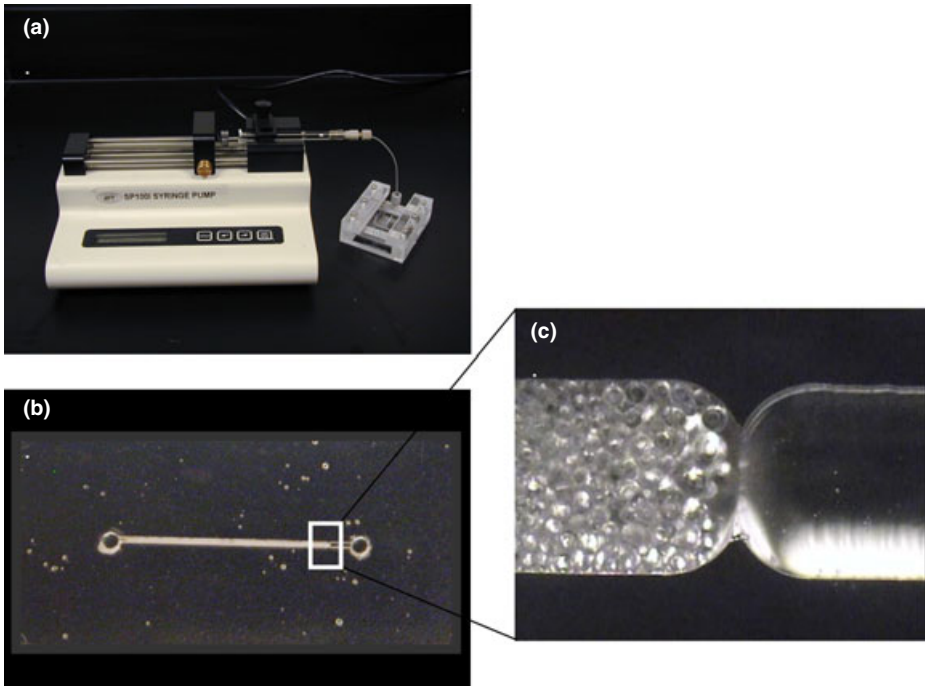

FIG. 5—*A microdevice setup for extraction of DNA from sperm cells. This includes a syringe pump, connected to the microdevice with narrow-bore tubing, and held in place by a homemade plexiglass mount (a). Silica beads 5–30 μm in diameter are packed against a weir (b and c) in the channel (200 μm deep, 420 μm wide) and adhered into place using sol-gel. (Figure reproduced with permission from Bienvenue et al., Journal of Forensic Sciences 2006, 51(2): 266–73. Copyright 2006 Blackwell Publishing, Inc.)*

toward analysis of sexual assault evidence. Also, excess DNA template added to the PCR can result in off-scale signal and other PCR artifacts. Bienvenue et al. (34) describe the use of a single-channel extraction device and modifications to the Breadmore et al. (33) cell lysis buffer to allow for on-chip sperm cell lysis and DNA purification (Fig. 5). The resultant DNA was of suitable purity and concentration for subsequent STR amplification, with comprehensive STR profiles generated. These results demonstrate the potential of microchip-based extraction methods for forensic analysis.

Another unique approach to microscale DNA extraction currently under development utilizes a serpentine channel design combined with an immobilized silica bead solid phase and fluidic oscillation. The method, developed by Chung et al. (35), relies on an immobilized silica solid phase. Instead of a solid column of packed phase, the silica beads were immobilized on the plasma-oxidized surface of the PMMA channels. Following bead immobilization, the solutions required for DNA binding, purification, and release were flowed back and forth through the device. By oscillating fluid flow over the immobilized phase, marked improvement of recovery and extraction efficiency over the same extraction methods with free beads was demonstrated. The method represents yet another microchip-based system for DNA purification with potential for forensic application.

The development of large-scale, commercial, SPE protocols enabled the facile translation of DNA extraction into microfluidic systems. The methods described here represent the first examples of microchip-based sample preparation for downstream sample processing and a major step toward the development of microfluidic systems capable of accepting crude samples for DNA analysis. As advancement of these technologies continues, the focus will likely turn from basic method development to the incorporation of extraction techniques with other microfluidic processes (cell sorting, DNA quantification PCR amplification, DNA separation and detection). The implementation of microfluidic DNA extraction for forensic analysis will allow the processing of a wide variety of samples and is an important component of future fully automated genetic analysis.

DNA Quantitation

DNA quantitation is a necessity in forensic DNA analysis, in contrast to most clinical analyses where the sample size is more consistent and DNA content more reproducible. Because of the predominance of samples containing low concentrations of DNA, quantitation must be carried out prior to PCR in order to minimize stochastic fluctuations that could cause allelic dropout from unequal amplification of the two alleles. For forensic use, DNA quantitation must be sensitive (mass detection limit on the order of picograms) and specific for human DNA.

To date, minimal effort has been directed at addressing pre-PCR DNA quantitation on microdevices. As demonstrated with a commercial microchip electrophoresis unit that uses an intercalating agent for detection, quantitation of DNA (typically PCR products) can be accomplished based on the separation and detection of known marker concentrations. While the limit of quantitation is ~50 pg/μL (36), it is not practical for forensic samples where DNA concentrations are often substantially below the detection limit. Direct quantitation of DNA on microdevices for forensic analysis will likely require the use of real time PCR which, conventionally, allows for quantitation of samples in the picogram to nanogram per microliter concentration range. Although PCR has been completed extensively on microdevices (as described in the subsequent section), the demonstration of quantitative PCR (qPCR) on microdevices is still in its infancy.

The first reported development of micro-quantitative PCR (μ-qPCR) was in 1998, for detection of viral, bacterial, and human DNA. Using silicon reaction chambers with thin-film heaters, Northrup et al. (37) demonstrated the use of both intercalating dye

(ethidium bromide) and 5′-nuclease (Taqman®, Applied Biosystems, Foster City, CA) detection methods. A subsequent paper (38) demonstrated detection limits of <10 pg in 40 cycles, comparable with commercial instruments. It is noteworthy that the μ-qPCR assay could be completed in ~35 min compared with 80 min for the conventional qPCR assay; however, the method used a reaction volume of 50 μL, a volume substantially greater than that used in many conventional methods. While these papers suggest proof-of-principle in glass microdevices, these silicon microchambers are not easily integratable with preceding and subsequent sample processing steps.

In 2002, a real-time μ-qPCR system was reported using silicon and Pyrex glass microdevices and SYBR Green I, a common conventional intercalating dye, in which 35 thermal cycles were completed in approximately 26 min (39). Owing to the length of the microchannels, the reaction volume was 25 μL, still much higher than many microchip (μ-PCR) methods. This microchip method for real time detection is promising, especially in its potential for integration with other sample processing steps that have previously been developed.

A two-step Taqman μ-qPCR assay was also demonstrated in 2002 by Quake et al. (40). The PDMS microdevice (shown in Fig. 6*a*) met the expectation of microminiaturization, using 12 nL reaction volume, combined with a flow-through approach to PCR, where the fluid was moved (via a microchannel) through two heated zones (denaturation and extension/annealing temperatures), resulting in 20–30 sec cycles. Figure 6*b* provides compelling evidence that the fluorescence profiles generated on the microscale and normalized against background produce the same type of curves typically obtained by real-time PCR with conventional macroscale instrumentation. Quantitation was not shown in the work, although appears to be feasible given a design incorporating multiple reaction chambers to generate a standard curve. While numerous other μ-PCR methods have been demonstrated (see PCR section below), this work accomplished the simultaneous amplification and detection of PCR product.

A μ-PCR method with electrochemical detection has also been developed that has potential for facile DNA quantitation (41). In this work, a hybrid silicon/glass microchip with 8 μL reaction chambers was used to accomplish asymmetric PCR, and the products simultaneously detected by hybridization of the amplified sequence to a probe-modified electrode—with the system, quantitation of the PCR product should be possible. Although the method would be effective for qPCR, the potential for widespread use is limited by the device that has integrated heaters, temperature sensors, electrodes, etc., thus increasing the cost of the device and making it less amenable to single-use disposability. While a reusable device may be acceptable in some clinical and research laboratories, disposability is anticipated to be paramount criteria in the forensic arena.

It is clear that much work remains in the development of effective DNA quantitation on microdevices. While a number of methods are currently under development, at the time of this writing, μ-qPCR appears to be the best fit for the needs of the forensic science community. The execution of quantitative PCR brings its unique challenges, most notably in terms of fluorescence detection in real time; however, it is anticipated that it will advance quickly and become commonplace in the near future. In addition, conventional qPCR methods permitting simultaneous male/total DNA quantitation or genomic/mitochondrial DNA quantitation should be fully extendable to microdevice methods.

PCR

Forensic DNA analysis was revolutionized with the advent of PCR-based STR profiling in the mid-1990s. Although a very powerful technique, the roughly three-hour PCR (for 30 cycles) significantly lengthens the overall analytical process. Hence the thermocycling required for the PCR process, perhaps more so than any other in the analytical sequence, could benefit by the transition to the microchip platform. Microchip PCR (μPCR) not only affords a much faster analysis time but also the opportunity for direct integration with DNA separation. Moreover, μPCR chambers have volumes on the order of nanoliters to microliters; thereby reducing the volume of reagents required and, consequently, has the potential to decrease the cost of forensic genetic analysis dramatically.

Cycling rates in most conventional PCR thermocyclers are hindered by the rate at which the block, and thereby, the tube and reaction mix, can be heated and cooled. Microchip PCR speeds temperature cycling when compared with block thermocyclers because the microchip substrate (glass or polymer) or reaction liquid (in the case of IR heating, as will follow) is heated, not a large block. In addition, hold times in conventional thermocycling (such as the commercially available STR kits) are on the order of 60 sec at each step as a result of the large reaction volume and the slow heating and cooling rates. Microchip methods offer the distinct advantage of significantly decreasing the reaction time by reducing the reaction volume by an order of magnitude or more. Pertinent literature is described below, although, for a detailed review of the wealth of literature on microchip-based PCR, see ref. (42).

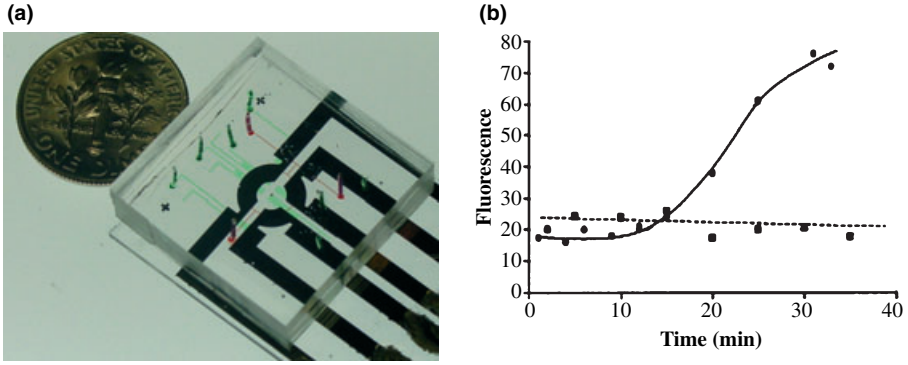

FIG. 6—*(a) Disposable PDMS rotary microchip utilized for real time PCR with a reaction volume of 12 nL. The fluidic channel is shown in red. Channels for fluidic control (valving, pumping) are shown in green. Resistive heaters (and electrical leads) appear as thick black lines. (b) Performance of a TaqMan® real-time PCR assay on the rotary μ-PCR device. Fluorescence intensity was monitored for both sample (●) and no template control (■). (Photograph (a) courtesy of Prof. Stephen Quake, Stanford University. Figure (b) reproduced with permission from Liu et al., Electrophoresis 2002, 23, 1531–6. Copyright 2002 Wiley-VCH Verlag GmbH & Co KG.)*

In 1998, a novel method of μ-PCR was demonstrated using a device with three heated zones, correlating to the annealing, extension, and dissociation temperatures of thermal cycling (43). The reaction mixture was flowed through each zone in one continuous channel. Hold times at each temperature were, consequently, based upon the flow rate of the mixture through the zones. In subsequent improvements to the flow through PCR method, Hashimoto et al. (44) reported PCR amplification of a 99 bp fragment of lambda DNA in only 1.7 min (20 cycles). Unlike many other μ-PCR methods, this method is not highly amenable to integration with other sample processing steps. Direct integration with other sample processing steps, such as the electrophoretic separation of PCR products is a hallmark of microfluidic devices and has been demonstrated in the literature (45–49).

Microchip PCR integrated with microchip electrophoresis (μ-PCR-ME) was first reported in 1996 by Mathies and coworkers (46). Unfortunately, the design of the PCR chamber made it impractical for further integration of processing steps on the front end, such as DNA extraction. In addition, large volumes (50 μL) were utilized for PCR, which have been shown subsequently by numerous laboratories to be unnecessary. Using a resistive heater, 30 sec cycles were achieved, with an overall assay time of approximately 15 min for 30 cycles. The entire process of DNA amplification and separation required approximately 45 min in the device (46). Importantly, however, in 2001, Mathies et al. (48) demonstrated μ-PCR-ME from single DNA templates. Although the stochastic effects at these low copy numbers are problematic for the forensic analyst, the work demonstrates that no loss in sensitivity is encountered by the transition to the microscale.

As an alternative to the contact heating described in all of the aforementioned methods, Oda et al. (50) developed the first totally noncontact heating method for PCR in capillaries using infrared radiation (IR). The IR light source was used to selectively heat the water in solution rather than the microdevice substrate, resulting in faster cycling times than with the Peltier and resistive heating methods. Initially, the work was completed in ~28 μL volumes, although subsequent manifestations of the method used nanoliter reaction volumes (51). A 240 sec PCR (15 cycles) was demonstrated in polymeric microdevices with a 2 μL volume using IR heating as a result of the well-matched thermal properties of the polyimide to the heating source (52). In borosilicate glass microdevices, Easley (53) demonstrated 25-cycle amplification of a 500 bp lambda phage DNA fragment in 5 min in a 130 nL reaction. This represents one of the fastest amplification reported to date and is approaching the biological limit of *Taq* polymerase. Unfortunately, fast amplifications represent only a portion of the challenge. Multiple PCR chambers (for simultaneous reactions) and multiplex amplifications (such as the STR kits) must also be developed to replicate the conventional technology.

On the macroscale, numerous PCR reactions can be performed simultaneously, as most conventional thermocyclers hold 24 or 96 polypropylene tubes. Therefore, to compete with conventional thermocyclers in terms of throughput, microdevices must allow for several reactions to be completed simultaneously, most importantly positive and negative controls. In the forensic arena, it also may be helpful if several items of evidence from a single case could be processed concurrently, therefore, multiple PCR chambers on a microdevice is an important consideration, particularly if the forensic DNA analysis microdevices were to be of a modular design (see Integrated versus Modular section below). The major limitation here is that uniform heating and cooling rates must be demonstrated in each chamber in order to ensure the fidelity and lack of bias in each amplification, which is a particular concern in some methods (e.g., IR heating) more than others. Simultaneous amplification in multiple PCR chambers on a single microdevice has been shown by Waters et al. (54) (four chambers).

In addition to multiple PCR amplifications on one device, for a PCR microchip to be viable in the forensics laboratory, it must be capable of amplifying multiple loci simultaneously, as accomplished using commercial STR kits. Multiplex μ-PCR has been demonstrated by Lagally et al. (45,47) and Waters et al. (55), through amplification of both amelogenin fragments, Legendre et al. (56) with AmpFlSTR® COfiler™ and Profiler™ amplifications (Applied Biosystems, Foster City, CA) on microdevices (Fig. 7), and Schmidt

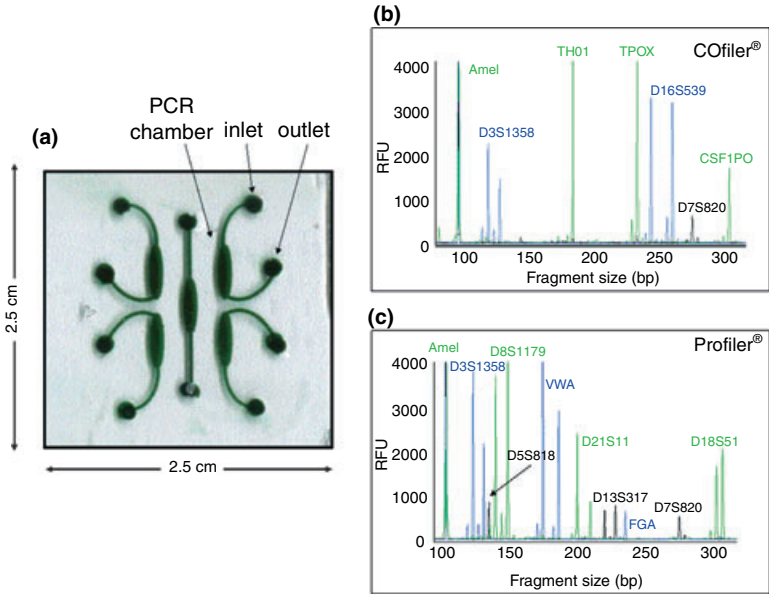

FIG. 7—*STR amplification on a glass microdevice. (a) An example of a microdevice for multiple STR amplifications on a single device. The chip contains five chambers: one for temperature control and four PCR chambers for parallel amplification (only one is labeled in the figure). Amplification of (b) Profiler® and (c) COfiler® multiplex STR loci was performed simultaneously on the microdevice using <1 ng template DNA. (Figure adapted and reproduced with permission from Legendre et al., Multiplex Microchip PCR for STR Analysis. Poster presented at The 15th International Symposium on Human Identification, 2004.)*

et al. (57) using PowerPlex® (Promega Corp., Madison, WI) 16 amplifications on microdevices in 1 μL reaction volumes. Legendre et al. (56) reported the first multiple loci STR amplification on a microchip, in only 200 nL. Thermocycling was completed on a conventional thermocycler in 100 min. No attempt was made to further decrease the cycling time, but there is every reason to believe that a move to an improved heating method would decrease thermocycling time significantly—in a manner similar to cycling times observed for other PCR amplifications on microdevices.

PCR has been a common pursuit among analytical microchip researchers, as demonstrated by the wealth of literature on the topic (42). However, for forensic DNA analysis, there remain a number of avenues for development. Extensive work has not been shown using the commercially available forensic STR kits or, further, multiple STR amplifications on a single device. When fully developed, however, microchip PCR will undoubtedly be a considerable time and cost savings to the forensic community.

DNA Separations

Forensic DNA profiling and genotyping is dependent upon the ability to separate STR sequences. Historically, these separations have been performed using electrophoresis in slab gels constructed of polyacrylamide (5). Although slab gels do allow for multiple samples to be run in parallel, there are drawbacks associated with these techniques. First, preparation is time and labor intensive, but more importantly, and fundamentally, slab gels are vulnerable to Joule heating, which limits the voltage that may be applied to the gel, thereby limiting the rate at which the separations may be accomplished. As a result of this limitation, capillary electrophoresis-based separations in fused-silica capillaries have become a viable alternative to the slab-gel format. The high surface-to-volume ratio inherent to the capillary (compared with that of the slab gel), more effectively dissipates Joule heating and, thus, allows high separation voltages to be applied, resulting in faster separation times. In addition, capillaries can be interfaced easily with online detection systems (laser-induced fluorescence, UV), permitting for high-resolution separations with rapid, accurate detection and sizing of alleles. As a result, capillary electrophoresis has become a widely accepted method for STR analysis.

Just as the forensic DNA analysis community witnessed the shift in technology from slab gels to capillaries in the mid-1990s, the translation of STR separations to the microchip format is anticipated, with its accompanying analytical improvements. Sample and reagent volumes needed in microchips are reduced further from that needed by capillaries, thereby decreasing the cost of each analysis and reducing sample consumption. In addition, faster separations may be achieved in microchips because higher separation voltages may be applied before encountering Joule heating (58). Multiplexed formats for DNA separations have also been demonstrated to allow high-throughput processing and, hence, truly contend with the conventional methods. Perhaps more significantly, however, microchips, unlike capillaries, afford the distinct opportunity to integrate other sample preparation steps of the forensic analysis (such as DNA extraction and PCR amplification) in an automated fashion [e.g., (59)].

For the development of any microchip system for STR separations to be successful, a number of requirements must be met. Naturally, these are highly dependent upon the ultimate design of the device (i.e., if the device is modular [high-throughput, single function] or totally integrated [multiplexed with other sample preparatory steps]). For multi-use, multi-sample separation platforms, a DNA sieving matrix of low viscosity is necessary so that it can be replaced between separations to ensure no carryover of DNA. In addition, the separation length must be adequate to obtain single-base-pair resolution while remaining compact enough to keep the device small. Also, to compete with the conventional capillary counterpart, the separations must be fast (<30 min) and multiplexable to allow for high-throughput analysis.

Compared with the wealth of examples of other DNA separations in the literature, few examples of true separations of STR products for forensic analysis in microchips have been demonstrated (60–67). More commonly, high-resolution DNA separation techniques have been developed in capillary for sequencing, STR typing, or other genotyping applications; however, these methods are often readily translatable to the microchip format. Development of a microdevice for high-resolution DNA separations necessarily focuses on finding a polymer with high-resolving power in a minimum separation distance.

For single-use devices, nonreplaceable sieving matrices, those that cannot be removed and refilled between runs, can be used to obtain DNA separations. These cross-linked gels are typically optically/UV transparent, electroneutral, and give better resolution than noncross-linked gels, but are not replaceable. Because of gel shrinkage and bubble formation over time, this relegates their applicability to primarily single-use devices. Photopolymerizable matrices, although nonreplaceable, such as those described by Ugaz et al. (68,69), may be advantageous for microfluidic systems as the nature of the polymer allows for precise placement within the device and high-resolution separation of DNA fragments. Accordingly, although not preferred for capillary-based systems, nonreplaceable sieving matrices may find more widespread application in microfluidic systems because of the potential disposability of the device.

In contrast to nonreplaceable systems, replaceable sieving matrices allow for the capillary or microdevice to be used repeatedly. One of the most commonly employed classes of replaceable sieving polymers used in the separation of STRs is self-coating polymers. These polymers rely on hydrogen bonding or hydrophobic interactions to adsorb to the wall and suppress electroosmotic flow, requiring no additional passivation steps to achieve high-resolution separations (70). Poly(vinylpyrrolidone) (PVP) (71), hydroxyethylcellulose (HEC) (72,73), and poly-*N,N*-dimethylacrylamide (PDMA) (7,72–74) were successfully utilized to separate STRs in capillary. It should be noted that PDMA is a component of Performance Optimized Polymer-4 (POP-4), the polymer utilized for STR separations in one commercially available capillary-based system (5). Because of their low viscosities, these self-coating polymers should be readily adaptable to the microchip format.

In addition to self-coating polymers, high-resolution DNA separations have also been accomplished using nonself-coating sieving matrices, such as linear polyacrylamides. These polymers require that the surface of the capillary or microchip be pretreated to suppress electroosmotic flow (EOF). To accomplish this, multiple preparatory steps must be undertaken that typically involve silanization to covalently bond the polymer to the surface (75). Linear polyacrylamide (LPA) was first used for DNA sequencing in 1993 and has become one of the most widely used replaceable separation matrices because of its low viscosity and long read length (76). Numerous DNA separations, including all of the reported STR separations that have been demonstrated on chip to date, use polyacrylamide as the sieving matrix. Using polyacrylamide, Schmalzing et al. (60) analyzed PCR product from an amplification of the CTTv multiplex in <2 min, demonstrating electrophoretic separations in 2.6 cm, but resolution of the 9.3/10 alleles of the TH01 locus was not obtained. By increasing the separation length to 11.5 cm and separation time to 10 min, resolution of these

alleles was achieved (61). Baseline resolution of all alleles in the PowerPlex® 16 amplification kit was reported by Mitnik et al. (63) in an 11.5 cm microchannel (35 min separation). In 2004, utilizing 4% LPA, Ehrlich reported the development of a 16-channel microdevice for STR separations (65). In the device, 16 simultaneous separations were completed in approximately 40 min with single-base-pair resolution and four-color laser-induced fluorescence detection, as demonstrated using a PowerPlex® 16 allelic ladder. However, the work utilized prepurification of the PCR product and separation times that are essentially equivalent to current CE methods, lending little impetus to move to the microchip methods. Mathies et al. (66,77–81) have also sought high-resolution DNA separations on microdevices, although primarily for sequencing reactions. In 2002, a 96-channel device (15 cm in diameter) capable of single-base-pair resolution up to 430 bp, which exceeds that necessary for the commercially available STR multiplexes, was developed (77). More recently, STR profiling was reported on a similar device, with the necessary single-base-pair resolution of the 9.3/10 TH01 alleles, in <25 min (66,79). Although STR separations have been demonstrated on microdevices utilizing linear polyacrylamide, it appears that shorter separation lengths (<10 cm) are not feasible with this separation matrix while maintaining the necessary single-base-pair resolution (82). It should be noted that, while the vast majority of the work reported is in glass microdevices, STR separations have been demonstrated on polymeric microdevices, as well (64,83). Shi et al. (83) separated Profiler Plus® STR fragments in ~18 min in a 10 cm separation length.

Poly(ethyleneoxide) (PEO), another nonself-coating polymer, has also been used to achieve single-base-pair resolution when separating STRs, although only on capillary-based systems (84,85). The PEO matrix is advantageous compared with POP-4, as the separations are faster, accomplished in shorter distances, and do not require high temperatures, all of which result in easier translation to the microchip format (84).

Although separation matrices are important for reproducible and well-resolved DNA profiles, they are not the only factor that must be taken into consideration when transferring capillary-based methodologies to microdevices. The separation distance required to obtain adequate resolution of STRs in the device is an extremely important factor to consider, as conventional capillary STR separations use a 42 cm capillary to obtain the needed resolution. In microdevices, the challenge is to achieve the same high-resolution separations, while maintaining a relatively small microchip footprint. Consequently, the focus of much research is on the development of novel channel geometries as a means to accommodate the long separation distances (and, hence, single-base-pair resolution) without greatly affecting the overall size of the microchip (86–89). Folding the fluidic channels (i.e., making serpentine channel designs) to reduce the size of microchips by introducing turns is one possible solution to the problem; however, simply creating a series of turns in the channel to maximize separation distance severely degrades resolution (90). The loss of resolution, due to a "racetrack effect," results from molecules on the inside wall travelling a shorter distance than those on the outside wall, but also at a faster rate, causing dispersion and loss of resolution (Figure 8) (87). In addition, electric field distortions created in the turns can contribute to a loss in resolution (91). A number of approaches have been developed to combat the dispersion, including the development of "tapered turns" (87), "constricted turns" (88) and "wavy turns" (89). The Mathies group has utilized a number of "tapered turns" in the design of the microdevice for STR separations (Figure 9B) and, thereby, have accommodated 96 separation channels into a microchip approximately the size of a compact disc.

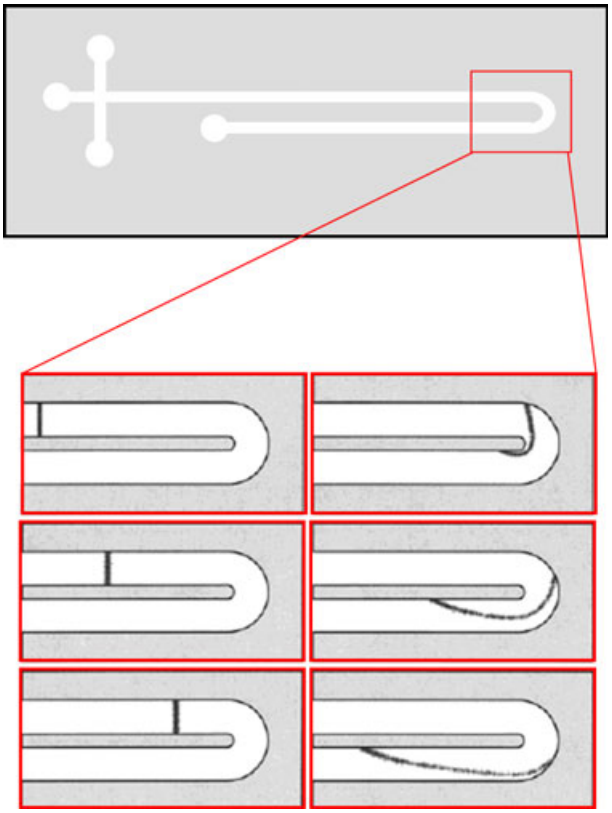

FIG. 8—*Simulation of the "racetrack effect." As molecules of the sample band traverse through the turn, the molecules on the inside of the channel not only traverse less distance, but are also subjected to band-broadening diffusion, causing a degradation of resolution and rendering such channel designs unusable for high-resolution forensic analyses. (Figure adapted from and reproduced with permission from Griffiths and Nilsson, Analytical Chemistry 2001, 73, 272–8. Copyright 2001 American Chemical Society.)*

Finally, to compete with slab gels and capillary instruments that can run many samples in parallel, a microchip-based platform for forensic DNA analysis must also be capable of high-throughput separations. A currently available commercial device, called GeneBench, is approximately 25 × 8 cm (~ 16 cm effective separation length) and simultaneously accomplishes 16 separations in up to 40 min [e.g., (67)]. Because the sieving matrix and channel designs have not been optimized to reduce the overall size of the device, however, it can hardly be termed a "microchip," as it is nearly 10 in long. In contrast, the Mathies group has developed a 96-channel micro capillary array electrophoresis (μCAE) device (Fig. 9*a*), in which STR fragments are separated on a 15 cm diameter glass wafer, resolving single-base-pair differences in under 25 min for up to 96 samples in parallel (66). Figures 9*c* and 9*d* shows typical electropherograms of the Promega PowerPlex® 16 allelic ladder and an STR profile (amplified with the PowerPlex® 16 kit) from the μCAE device, where it is evident that the necessary resolution is obtained. Yeung et al. (79) successfully demonstrated the functionality of the device on nonprobative casework samples.

The genesis of the analytical microchip is rooted in separations; consequently, it is natural that their use in DNA separations is the most advanced of all sample preparation steps described here. As a result, the DNA separation module has been the first aspect introduced into forensic laboratories. The μCAE device is currently being evaluated at the Virginia Department of Forensic Sciences (66). The ability to achieve high-throughput DNA separations in microdevices makes these microchip-based systems as efficient as

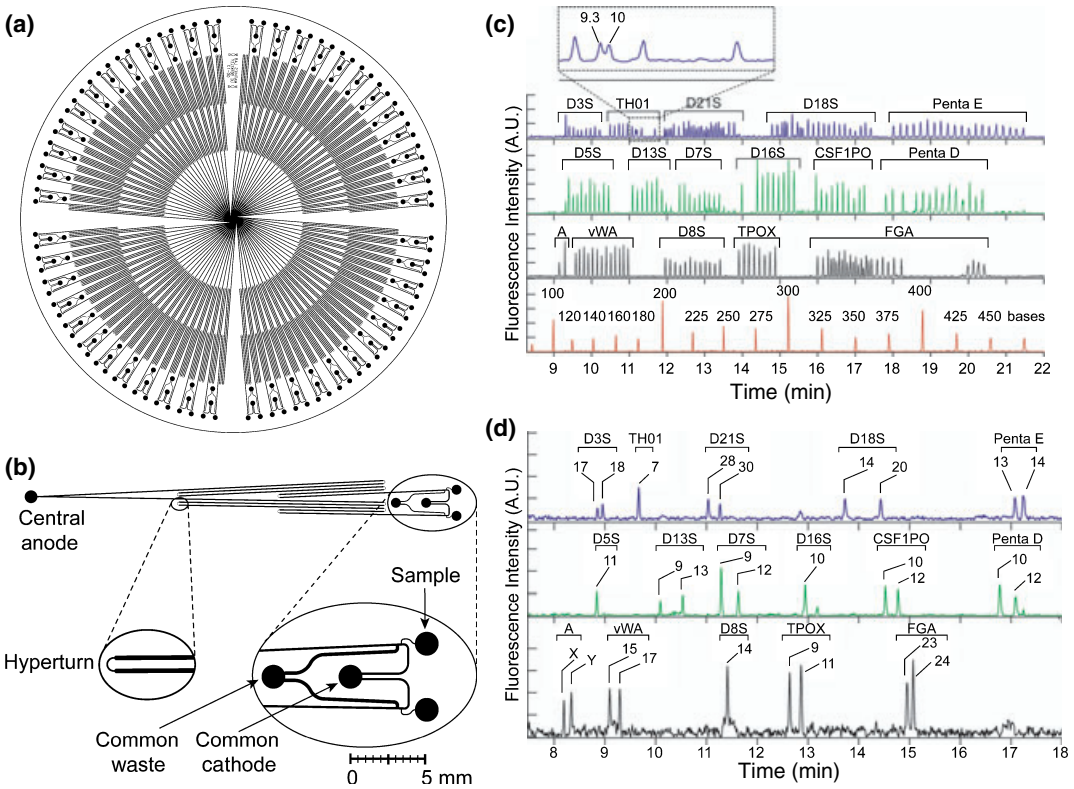

FIG. 9—*(a) Design for the 96-channel radial capillary array electrophoresis microdevice by Mathies and coworkers. The device is comprised of 48 doublet structures, each containing two electrophoresis lanes that share common cathode and waste reservoirs. (b) Magnified view of the doublet structure, highlighting the "hyperturn" (also called "tapered turn"), which prevents band-broadening dispersion as the DNA molecules traverse the turn, thereby enabling high-resolution DNA separations in a compact device. Electropherograms of (c) Promega PowerPlex® 16 allelic ladder and (d) an STR profile obtained from 0.17 ng template DNA amplified using Promega PowerPlex® 16 obtained on the micro capillary array electrophoresis (μCAE) microdevice. (Figure adapted and reproduced with permission from Yeung et al., Journal of Forensic Sciences 2006, 51(4), 740–7. Copyright 2006 Blackwell Publishing, Inc.)*

current multiplexed capillary systems and, thus, a viable alternative to existing separation technologies. As a result, widespread use of these devices in forensic laboratories for STR separations is anticipated in the near future.

Integrated Versus Modular

In developing microdevices for forensic applications, a number of issues must be addressed with respect to the overall design of the devices. Included in these is whether the system should be "*modular*" (different chips for different sample preparation methods and for analysis) or "*integrated,*" with sample preparation seamlessly mated with analysis on the same device in a way that was originally conceptualized for μTAS or LOC systems.

LOC systems with "sample-in-answer-out" capabilities have been promised now for more than a decade. Only recently has a microdevice with this capability been demonstrated using clinical applications (59). The device begins with introduction of <1 μL crude sample and incorporates DNA extraction and purification, PCR amplification, and DNA separation in a seamless fashion. In one example, anthrax infection was detected in mouse blood with a total analysis time of <25 min. Similarly, an integrated microdevice for forensic DNA analysis can be envisioned. Figure 10 illustrates a basic design for a totally integrated microchip that would be used for forensic analysis of vaginal swab evidence, as foreseen by our laboratory. All of the processes required for STR analysis of DNA are incorporated into the device, allowing sample introduction at one point with STR results collected at the

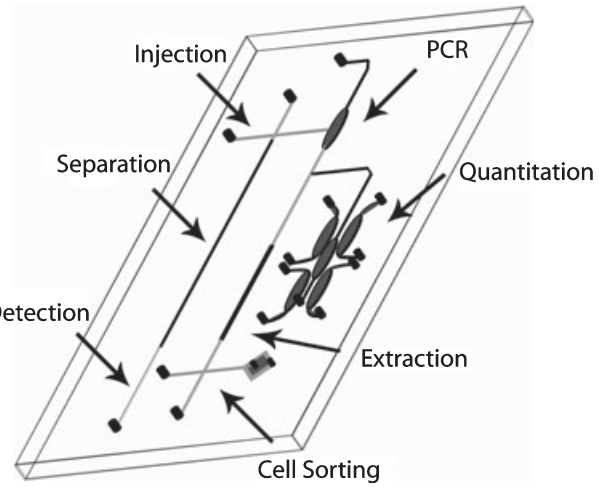

FIG. 10—*Prospective design for a totally integrated microdevice for forensic DNA analysis of sexual assault evidence. The device would be capable of accepting biological material desorbed from a cotton swab and processing the sample through various domains (highlighted) that would accomplish sequential cell sorting, DNA extraction, quantitation, amplification, and separation of STR products in an automated fashion, providing a fully functional μTAS for forensic genetic analysis. A design such as the one pictured, would allow for sample processing without user intervention at each of the analysis steps, eliminating multiple entry points for contamination and providing a contained and controlled analysis system.*

detection point. A distinct advantage of an integrated system is the minimal manual sample handling, which diminishes opportunities for laboratory sources of contamination. However, a totally integrated system requires fabrication of additional features and significant complexity into the microchip and requires precise control of fluidics in various regions of the chip, while remaining cost-effective for disposability.

An alternate manifestation of microchips in forensic laboratories is the modular system, where separate devices may be used for differential extraction/DNA extraction, DNA quantitation and amplification, and STR separation. Modular devices provide a number of characteristics that are particularly advantageous for forensic analysis, including the opportunity to isolate each processing step on a different microchip. While this requires less fluidic control, the seamless (and efficient) transfer of material from one module to the next must be addressed. Additionally, the modular design provides the flexibility of using different microchip substrates for each module, particularly those best suited to the process (i.e., plastic for PCR, glass for separations, etc.). They also offer the opportunity to store evidence on-chip at virtually any (or all) steps of the analysis for further processing at a later date. Finally, the modular design (e.g., Fig. 11) provides more flexibility for multiplex analysis of samples for high throughput applications.

The advancement of DNA technology to microdevices affords forensic scientists with a yet unaddressed issue—to what extent should the portability of these devices be harnessed? That is, should a totally integrated device (with accompanying instrumentation) be used in the field in order to obtain profiling results rapidly and, thus, aid in investigative decision-making? Alternatively, should a sample-preparation module be utilized by crime scene technicians to store a probative sample for subsequent analysis in the laboratory? These, among other issues, result from the new technology and must be addressed by the forensic community in parallel with its development.

In both modular and integrated designs, disposability of the devices will be necessary in most instances. That is, any sample preparation modules would likely be deemed single-use, although the separation module may be designed for repeated use, in a manner analogous to the current capillary-based methods used for STR product analysis. While each design has its inherent pros and cons, the forensic community is at a unique juncture to help shape the future research directions by making their opinions and needs known with regard to the ultimate manifestation of a microdevice for forensic DNA analysis.

Other Forensic Device Applications

The scope of semi-automated evidence processing on microdevices is not limited to STR analysis. Single nucleotide polymorphism (SNP) analysis of forensic DNA evidence has also garnered attention in the forensics community (92,93); however, a more thorough discussion of the technology is outside the scope of this review. In brief, SNP analysis can be achieved by numerous means, including hybridization arrays (94), and while these are commercially available (primarily for basic biomedical research), they are not routinely integrated with sample preparation steps, a major benefit of microfluidic systems. Other more classical microfluidic approaches of SNP analysis, likely lower in throughput capability, have been demonstrated as well (95,96). Additionally, the analysis of RNA on microdevices is foreseeable as well. Although few examples of microfluidic analysis of RNA exist (97–100), it is easy to envision the application of microfluidic devices to the purification and reverse transcriptase PCR amplification of RNA. Disposable, self-contained microchips may even enable a more efficient analysis of nuclease-susceptible RNA, preventing contamination and preserving a sterile environment for these applications.

The reach of microchip technology to forensic analyses beyond nucleic acid evidence is becoming increasingly evident. In the area of illicit drugs and toxicology, microchip assays have been developed for blood alcohol testing (101) as well as separation and identification of amphetamines (102), barbiturates (103), and tricyclic antidepressants in serum (104). With the latter two examples, the microdevices were designed for detection by electrospray ionization mass spectrometry. Microdevices for use in counterterrorism efforts are also currently under development, including the detection of explosives (105–109), chemical warfare agents (110,111), and biowarfare agents (33,112,113). See reference (108) for a focused review of the literature. These applications of microchip separations, in combination with miniaturized mass spectrometry (114), make confirmatory identification of these analytes possible in the field. It is foreseeable that any sample processing steps prior to separation, such as SPE of small molecules, can be integrated into a microdevice for automation of the entire process. A number of research groups are utilizing microchips in this manner (115–117), similar to the integration of DNA extraction, PCR, and DNA separations highlighted above. (59)

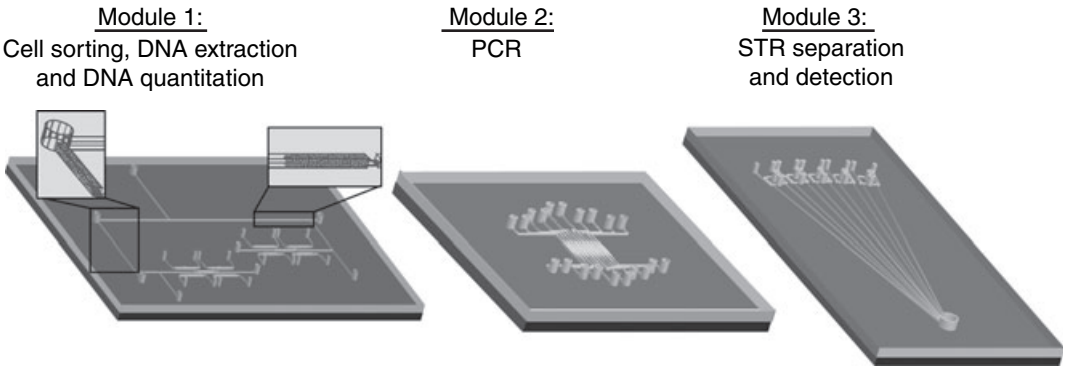

FIG. 11—*Proposed modular microdevices for forensic DNA analysis. In this manifestation, the sample processing steps are divided into three modules: (1) Cell sorting/DNA extraction/quantitation, (2) PCR, and (3) STR separation. Seamless interfacing of these modules would be required for total analysis to be accomplished, necessitating microfluidic interconnects between each device. The modular design would allow for easy storage of the biological material at each step and may allow a slower conversion to microfluidic technology by permitting the introduction and implementation of the devices into crime labs in a stepwise fashion. A modular device design, however, may require more user intervention and also provide more entry points for potential contamination.*

Conclusions

The rapid development of microdevices over the past decade has forged the way for their successful application to forensic genetic analyses. As improvements to the individual microscale sample processing steps, such as DNA purification, amplification, and separation for both clinical and forensic purposes continues, the potential of microfluidic systems for genetic interrogation has become increasingly more evident. Because of the potential impact of microchips on the forensic community, scientists have recently begun to address the unique caveats inherent to the analysis of forensic samples. As a result, the application, development, and validation of these devices for forensic DNA analysis has grown tremendously and leaves little doubt that a paradigm shift is occurring in forensic DNA testing.

A number of scientific and engineering hurdles must be overcome before microfluidic devices can become universally implemented in forensic laboratories. Foremost, there is a need to effectively bridge the gap between the macroscale and microscale regimes. More specifically, the front end of the device must be able to accommodate "large" (hundreds of microliters) volumes for input of the biological material, whereas the volumes typically manipulated on chip are <1 μL and, for separations, <1 nL. For instance, in a sexual assault case, biological material is typically eluted from a vaginal swab in approximately 500 μL. Therefore, the microdevice must be able to accommodate the entire volume and concentrate the purified DNA into nanoliters to be directed to the PCR chamber for amplification in a total reaction volume of <1 μL. While the volume (and, thus, the associated thermocycling time and reagent consumption) required for the PCR amplification has been dramatically reduced on-chip when compared with the conventional process, there has not been sufficient research to determine if a subsequent reduction in the mass of DNA required will follow. Reports (48,118–121) have demonstrated single-copy (and other low copy number) DNA amplification on microdevices, although nothing has been reported with regard to limits of detection or stochastic effects when amplifying STRs on-chip. The concentrating effect on the microscale (with the same starting mass of DNA) suggests that fewer starting copies can be amplified before encountering stochastic effects such as allelic dropout. However, only through further evaluation will it be known whether implementing microdevices in forensic laboratories will diminish the amount of evidentiary material needed or consumed.

While the final manifestation of microdevices in forensics is not yet clear, it is likely that microdevices will be the next technology platform encountered in forensic DNA laboratories. Modular, single-process devices and totally integrated microfluidic systems are in development to fill both high-throughput batched and complete single-sample analysis niches. One could envision high-throughput devices to be more advantageous for large DNA caseloads, whereas totally integrated devices may be preferable for crime scene testing or in high-priority cases that require rapid results. Commercialization of these systems has only just begun, with DNA separation devices already available; however, with the successful validation and implementation of these microfluidic platforms in crime labs, it is anticipated that other sample-processing modules and integrated microfluidic analysis systems will not be far behind. While the eventual cost of these microdevices is, understandably, important to the forensics community, it is impossible to predict at the current time, as the cost will depend upon the substrate utilized, the complexity of the devices (including integrated vs. modular designs), and the market. The flexibility of microfluidic device design makes their application to casework analysis both in crime labs and at the crime scene a viable alternative to current methodologies and represents a revolutionary change for evidence processing and handling. Furthermore, we believe the speed and automation that accompanies the technology transfer from the conventional, macroscale processes to the microscale analytical methods stands as a viable solution to the DNA casework backlog in forensic laboratories. While robotics can, undoubtedly, result in greater automation in the laboratory, there is no inherent decrease in the processing time of the component steps, resulting in only an incremental advantage over the conventional methods. With the development, validation and implementation of microfluidic devices for forensic DNA evidence, forensic scientists would be armed with a rapid analysis technology that could radically change the established approach to DNA analysis by enabling more efficient casework processing.

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