High Speed STR Analysis on Microfabricated Electrophoretic Devices

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ABSTRACT

In this paper we summarize early results of a microdevice which has the potential to revolutionize several aspects of the Short Tandem Repeat (STR) method through a 100X increase in the speed of analysis, while simultaneously allowing the technique to become both fully automated and sufficiently miniaturized to become portable. The approach should reduce the cost of STR analysis by about one order of magnitude and permit entirely new applications in high-speed portable forensics.

Specifically, the proposed apparatus has been shown to perform the STR electrophoresis assay in as little as 1-2 minutes on a device measuring several inches. This compares to conventional electrophoresis systems which requires 1-3 hours on large, non-automated machines. The speed of the chip approach is highly valuable as a means to accelerate time-critical case work.

The performance of eight channel microfabricated quartz electrophoretic device is investigated with the CTTv quadriplex short tandem repeat allelic profiling system. The separation efficiency matches the predicted performance indicating that the multi-channel device functions properly. A throughput of 384 samples per 8 hour day is predicted for the device after further automation is implemented, and even higher throughput is envisioned for higher density devices.

INTRODUCTION

The short tandem repeat (STR) system provides a highly discriminatory method of linking human identity to DNA evidence. Over the next several years, the State and National forensics laboratories will face the challenge of assembling and applying convicted-felon databases operating on this method. These laboratories will then be responsible for enlarging STR databases as required by statute and applying the method to time-critical cases within the criminal justice system. At the same time the Human Genome Project and other applications of DNA assays have emphasized the need for non-traditional (non-slab-gel-based), high-speed, high-throughput, low-cost DNA analysis methods (1,2,3). One solution to this serious technical challenge has been the use of miniaturized electrophoresis devices fabricated in glass, fused silica or plastic, containing microchannels of 20 - 100 µm i.d. (4, 5). Several potential advantages are associated with such devices. First, there is high analysis speed which is due to high electric field strength, very narrow injection plugs and short separation distances. Second, very high-throughput appears to be possible, since multiple miniaturized channel structures can easily be incorporated on a single wafer and simultaneously operated. Third, a substantial reduction in overall analysis costs is expected since only minute amounts of expensive reagents are consumed per analysis and because total automation of the entire micro-electrophoretic analysis process seems to be feasible. Several applications of such devices to DNA analysis have been reported (6-9). Specifically, our group has demonstrated that the quadriplex STR system CTTv can be analyzed in less than two minutes in a microfabricated device with less than 3 cm separation distance (10). This is a factor of 10 and 100 times faster than capillary or slab gel analysis, respectively. In addition, we recently demonstrated that approximately 400 bases can be sequenced in only 14 minutes in a 11.5 cm long microchannel (11). The present paper will focus on the throughput aspect of such devices. In particular, high speed analysis of multiple STR samples (12) on a single microfabricated device will be investigated.

MATERIALS AND METHODS

Micromachining. Electrophoretic microchip devices were fabricated as described in previously (10) utilizing standard photolithographic and chemical etching methods to produce 40 µm deep channel structures in fused silica wafers. The channels have a nearly half cylindrical shape resulting in a cross sectional area equivalent to that of a cylindrical capillary with an internal diameter of 70 µm.

Coating. The surfaces of the etched channels were chemically modified by covalent attachment of linear polyacrylamide as reported (13) to stop electro-osmotic flow and to prevent non-specific interactions.

Separation Matrix. The working buffer consisted of 1 X TBE with 3.5 M urea and 30 % v/v formamide (Pharmacia, Uppsala, Sweden). The separation matrix was 4 % (w/v) non-crosslinked linear polyacrylamide synthesized in-house by redox polymerization (10). The

matrix was stored at 4 °C and loaded with a syringe into the coated microchip channels.

Instrumentation. The eight channel microdevice was mounted on a stage fitted with high voltage connections and optical access for laser induced fluorescence (LIF) detection. The voltages applied to each reservoir were controlled by a manual switching circuit and resistor based voltage divider network. Actual applied voltages were verified with a Keithley Instruments 614 electrometer with a high voltage probe. Laser-induced fluorescence was achieved using an argon ion laser operating with 4 mW of output power at 488 nm. The beam was focused to a spot size of 15 μm in the channel at 30 degree angle of incidence with a 10 cm focal length lens. A 50X 0.45 N.A. long working distance microscope objective (Bausch & Lomb) collected the fluorescence emission. The collected light was spatially filtered by a 4 mm diameter aperture in the image plane and optically filtered by two 520 nm bandpass filters (520DF20 Omega Optical, Brattleboro, VT) and detected by a photomultiplier detection system. The PMT current signal was converted to voltage across a 10 kohm resistor, digitized with a PC-controlled 20 bit data acquisition system (Data Translation model 2802, Marlborough, MA) and analyzed using C Grams software (Galactic Industries, Salem, NH).

Microchip Gel Electrophoresis. The eight channel chip, freshly filled with the 4 % (w/v) polyacrylamide solution, was pre-electrophoresed for 3 min across the separation channels at 200 V/ cm and 50 °C. Separations were carried out under identical electrophoretic conditions. The chip was operated in the pinched cross injection mode (14), simultaneously injecting in all eight channels and producing in each channel an injection plug length of 100 μm and an injection volume of 0.3 nL.

The STR samples consisted of 2 μL of CTTv standard ladder (Promega, Madison, WI) diluted to a final volume of 10 μL with formamide. They were briefly vortexed, denatured for 2 min at 95 °C, chilled on ice. Eight STR samples were loaded four at a time in parallel into the sample reservoirs using an ordinary 4-way pipettor.

RESULTS

Figure 1 depicts the structure of an eight-channel microfabricated array tailored to the specific requirements of the simultaneous high speed analysis of eight STR samples. The separation channels are fanned out at the top of the device to make space for the reservoir structure which match the spacing of a standard 96 well plate. They are packed together at the bottom to keep the detection scanning distance narrow. All eight separation channels have essentially the same lay-out consisting of a simple cross-structure, derived from our previously described single channel STR device (10). Each separation channel has its own independent sample port whereas waste ports are shared whenever possible to economize for space. All separation channels are connected together at top and bottom not only to allow for a common anodic and cathodic buffer port but also to permit a convenient single-step replacement of the polymeric sieving matrix in the entire channel system. The separation distance between injection point and detection point is only 45 to 50 mm. We have previously shown, both by experiments and through theoretical considerations, that such a short separation length should suffice for reliable high speed STR analysis (10,11). In a typical analysis sequence the polymeric sieving material is loaded and the reservoirs are filled with buffer. Sieving matrix exchange is performed after each run from the anodic end with a detachable mechanical fixture holding a syringe filled with the separation matrix. The microdevice is then put into place where proper electrical and optical contact is made with the device. After a few minutes of thermostating to 50 °C and pre-electrophoresis, 8 STR samples are pipetted into the sample reservoirs (the reservoirs are spaced such that 4 samples at once can be loaded). The samples are simultaneously cross-injected into the separation channels and after a short separation time would ideally be simultaneously detected at the anodic end of the separation channels by a laser-induced-fluorescence scanning detection system. Figure 2 shows representative electrophoretic profiles for the CTTv standard ladder (consisting of the four loci vWA, TH01, TPOX, CSF1PO) generated in the described eight-channel device. Eight STR samples were injected simultaneously but were detected individually by a single point detector which was placed on a different separation channel for each run. The fast separation times of under 3 minutes and the analysis profiles are virtually identical with those we previously achieved on a single channel structure of comparable dimensions indicating that our eight-channel device functions properly. Moreover, we have good experimental evidence that the described analysis cycle can be repeated many times before the microdevice has to be replaced due to chemical deterioration of the channel surface passivation or other failures (e.g. sample cross contamination).

CONCLUSIONS

We have demonstrated that it is feasible to perform simultaneous genotyping analyses at high separation speed and with good resolution on an eight-channel microdevice. At present, the device is operated manually but work is under way to fully automate the entire analysis sequence and to implement a scanning detector.

This very conservative eight channel system would process 384 samples per eight hour shift, assuming a turn around time of ten minutes when fully automated with robotic sample loading and reagent replenishment. A more aggressive 96 lane device would handle 4068 samples in the same 8 hour shift, which would meet the demands of most centralized laboratories or moderately large population screening studies.

The microdevice STR system will also generate a *high-speed* capability that will produce order-of-magnitude faster (virtually real-time) STR results for time-critical case work. This latter capability has been fully demonstrated in multiplexed microchip STR analysis in our laboratory.

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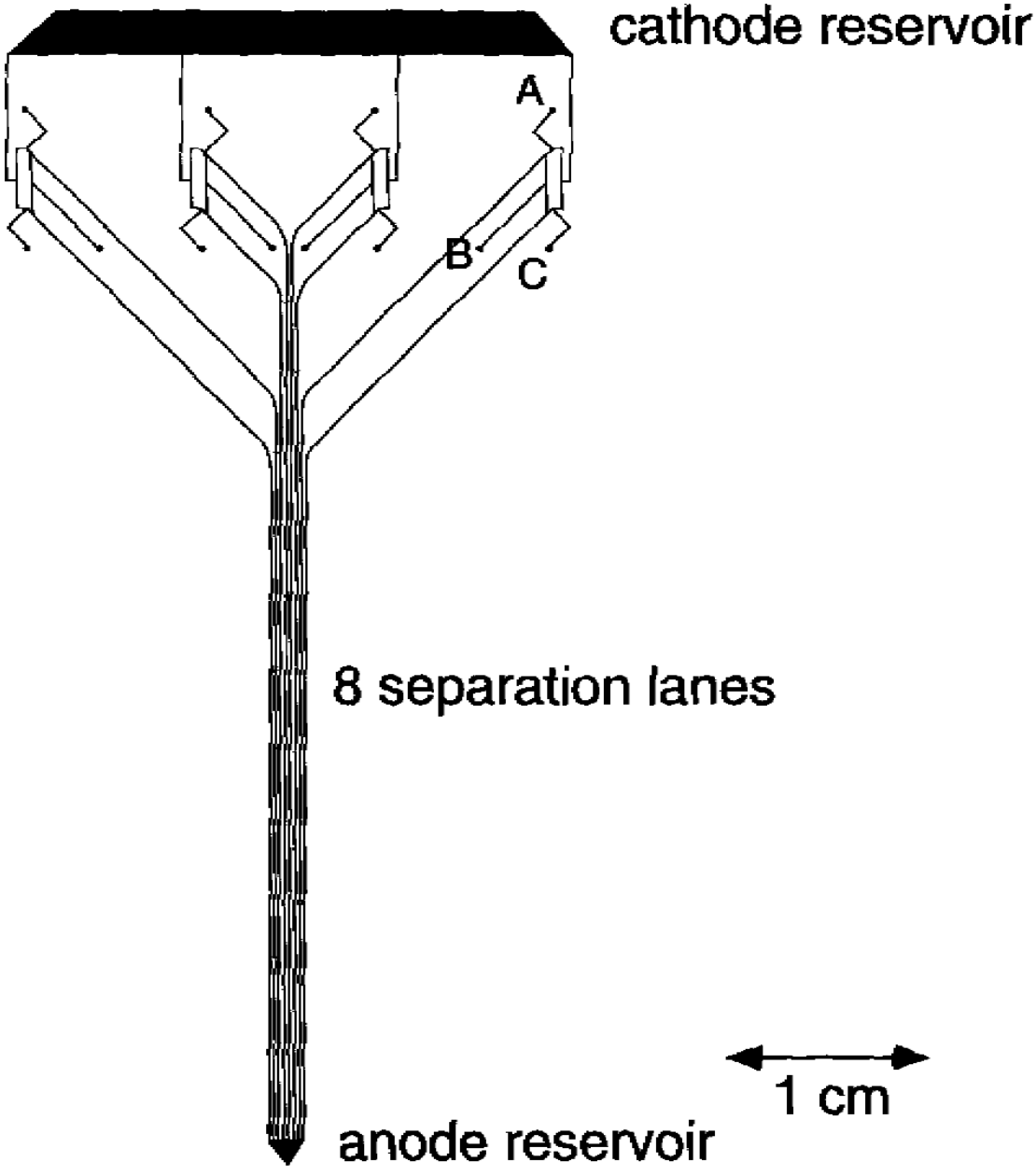

Figure 1. Schematic of the eight-channel genotyping device microfabricated from fused-silica wafers. The channels have an effective separation distance of 45 to 50 mm and their internal cross-section equals that of a 70 μm i.d. circular capillary. In the figure A and C denote sample reservoirs, and B denotes their shared waste reservoir.

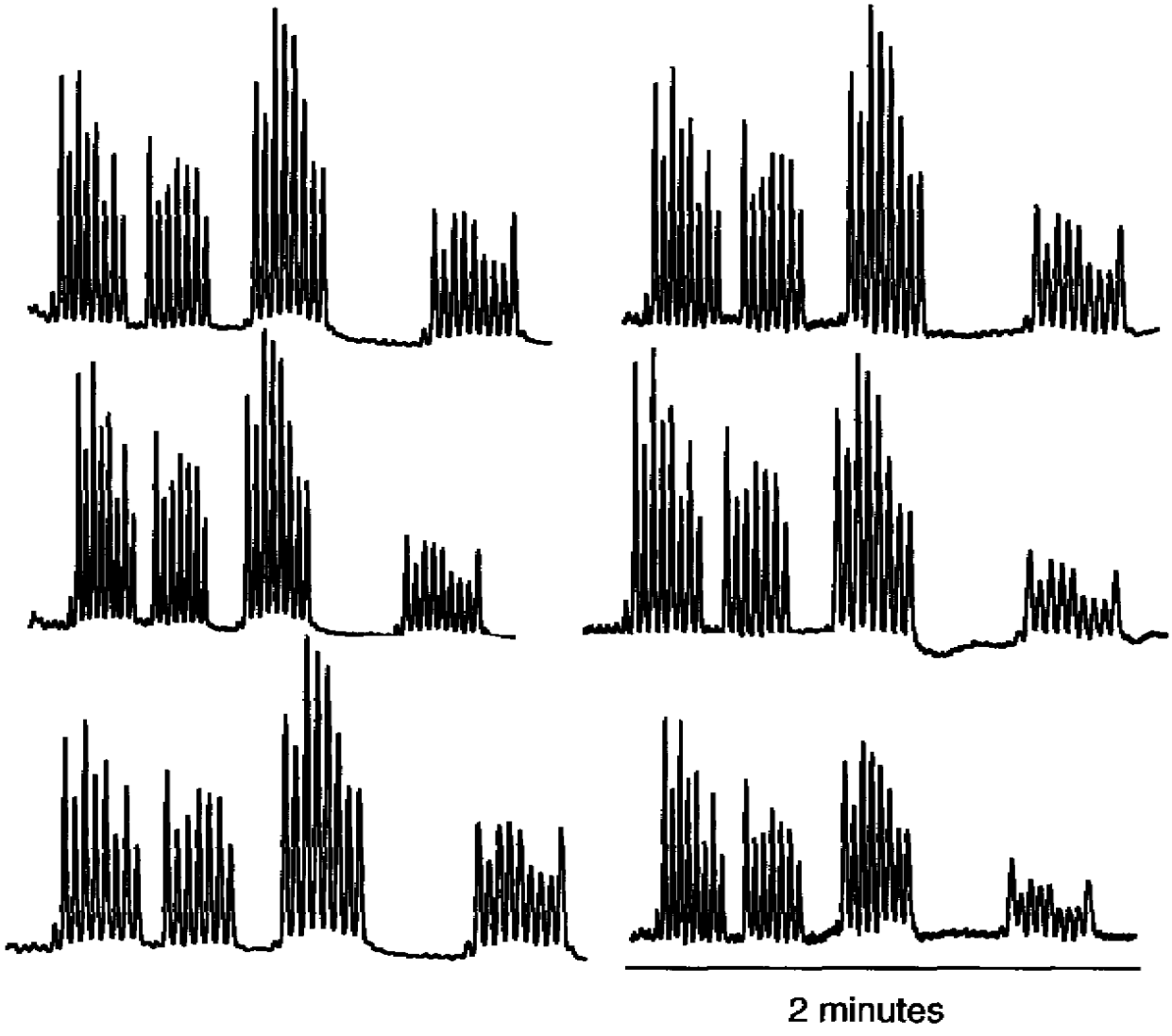

Figure 2. Multi-channel genotyping results. Samples of the quadriplex STR standard ladder CTTv were pipetted into the eight sample reservoirs of the top plate and then simultaneously cross-injected for 2 minutes at 200 V/ cm. The separations were performed at 200 V/ cm, 50 °C in a 4 % replaceable linear polyacrylamide separation matrix in denaturing buffer. The samples were detected individually with a single point induced fluorescent detector between 45 to 50 mm below the injection point.