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An accessible micro-capillary electrophoresis device using surface-tension-driven flow

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Abstract

We present a rapidly fabricated micro-capillary electrophoresis chip that utilizes surface-tension-driven flow for sample injection and extraction of DNA. Surface-tension-driven flow (*i.e.* passive pumping) injects a fixed volume of sample that can be predicted mathematically. Passive pumping eliminates the need for tubing, valves, syringe pumps, and other equipment typically needed for interfacing with microelectrophoresis chips. This method requires a standard micropipette to load samples before separation, and remove the resulting bands after analysis. The device was made using liquid phase photopolymerization to rapidly fabricate the chip without the need of special equipment typically associated with the construction of microelectrophoresis chips (*e.g.* cleanroom). Batch fabrication time for the device presented here was 1.5 h including channel coating time to suppress electroosmotic flow. Devices were constructed out of poly-isobornyl acrylate and glass. A standard microscope with a UV source was used for sample detection. Separations were demonstrated using Promega BenchTop 100 bp ladder in hydroxyl ethyl cellulose (HEC) and oligonucleotides of 91 and 118 bp were used to characterize sample injection and extraction of DNA bands. The end result was an inexpensive micro-capillary electrophoresis device that uses tools (*e.g.* micropipette, electrophoretic power supplies, and microscopes) already present in most labs for sample manipulation and detection, making it more accessible for potential end users.

Keywords

Liquid phase photopolymerization; Microelectrophoresis; Passive pumping

1 Introduction

Development of micro-capillary electrophoresis chips has been an area of active research for over a decade [1-3]. Miniaturization of electrophoresis has many advantages including reduced material usage, compact analysis space, and high-speed analysis. Since the first reports of microchip electrophoresis, steady improvements have been made on the technology. Analysis time has decreased steadily reaching the point where certain separations can be performed on the order of minutes [4,5] depending on the type of analysis. Increased functionality has been integrated into microelectrophoresis chips in the form of on-chip fluorescent detectors [6,7] and electrochemical detectors [8]. Micro-capillary electrophoresis has also been successfully integrated with lab-on-a-chip platforms where raw biological samples are injected into a

microchip, DNA is extracted, processed *via* PCR, and then separated [9]. While the majority of research has focused on improving the efficiency of electrophoresis analysis, less attention has focused on making such devices more accessible to the end user. For example, life science researchers perform electrophoretic separations routinely. Typically these separations take hours to conduct if they are done in gels or are expensive if using a commercial capillary electrophoresis machine. In addition, circumstances arise where only a small amount of a rare sample is available for testing, making it difficult to use traditional methods for analysis. Life science researchers could benefit from a decrease in analysis time and reduced sample usage that micro-capillary electrophoresis chips offer if they were more accessible at a reduced cost. It should be noted that a current commercial micro-capillary electrophoresis system (Agilent 2100 Bioanalyzer) has been successfully integrated in high-throughput settings or commercial labs providing reduction in time and reagent use. However, the start-up costs are high (\$18 000 for a base unit) and more than many academic life science labs are willing to spend. The device presented here is aimed at providing an inexpensive micro-capillary electrophoresis chip to researchers with minimal costs by using materials and equipment already available in most life science labs. Thus, in general, two issues in particular prevent micro-capillary electrophoresis devices from being accessible. One is the cost of the device and second is the complexity of device operation.

Microelectrophoresis devices reported in literature are typically made of quartz [10], glass, PDMS [11-13], polycarbonate [14], and PMMA [15]. While each of these materials has advantages and disadvantages from a manufacturing and prototyping point of view, the materials and associated fabrication methods also make microelectrophoresis chips expensive and difficult to fabricate without specialized equipment. Additionally, micro-capillary electrophoresis chips are not readily available off the shelf at an economical price, further limiting the number of life science labs that use them.

As mentioned previously, the operation of microelectrophoresis devices makes them less accessible to life science labs since specialized liquid handling equipment (*e.g.* syringe pumps, pneumatic pumps, valves) are required for reliable sample introduction and extraction. Due to the short separation distances and separation times, a small reproducible plug is needed to produce consistent retention times and peak areas in a given device. Traditionally, this is achieved *via* electrokinetic or pressure/pneumatic injection schemes. Electrokinetic injection is one of the most common ways to inject a sample into a microchannel. In a typical setup, this method takes advantage of the charge present in the sample and an applied voltage drives the sample towards an intersection for delivery into a separation channel. Pneumatic injection uses pressure and valves to deliver sample plugs into a microfluidic device. Both methods have proved to be viable schemes for sample delivery. However, employing these methods is complex and expensive. Electrokinetic injection also suffers from two bias problems: (i) bias due to the conductivity of the sample, which can affect how much sample is injected and (ii) bias due to mobility differences among solutes, which causes highly mobile solutes to be preferentially injected compared with lower mobility ions. While methods have been developed to compensate for these phenomena, it does add complexity to the injection scheme. A complete review on injection methods for micro-capillary electrophoresis chips can be found by Wenclawiak *et al.* [16] and Roddy *et al.* [17]. In this work we present a simpler method for sample injection that leverages surface tension effects that dominate on the microscale to deliver fixed volume samples of DNA for electrophoretic separation. This method called “passive pumping” requires no complicated setup of tubing, pumps, and power supplies associated with typical injection methods, making it possible for microelectrophoresis devices to be more accessible for general use. The equipment required for passive pumping injection is a micropipette, found in most laboratories, and a hydrophobic surface to manipulate surface tension for pumping. We also demonstrate sample extraction from the device using passive pumping. A comparison of these sample manipulation methods is presented in Table 1.

Previously we published work describing a method for fabricating gel-based microelectrophoresis devices using liquid phase polymerization that can be done without the need of a cleanroom and the costs associated with such facilities [18]. Microchannels were constructed within a sealed chamber in minutes as opposed to the hours required for more traditional methods. The micro-capillary electrophoresis device presented here is an extension of this work. Here we present an inexpensive methodology for constructing micro-capillary electrophoresis chip that utilizes passive pumping for sample injection/extraction. The purpose is to utilize equipment already present in typical life science labs such as micropipettes, electrophoretic power supplies, and UV microscopes, with the micro-capillary electrophoresis chip. This allows the technology to be more accessible for the potential end user as no additional materials are needed to run the device.

2 Theory

Passive pumping is a method of producing fluid flow *via* surface tension effects [19]. The pressure within a droplet of a small radius is higher than a droplet of the same fluid with a larger radius. This is described by the Young–Laplace equation, which relates surface tension, the radius of curvature of a drop, and the gauge pressure within the droplet, $P = 2\gamma/R$. Thus, when droplets of different size are connected by a microchannel, the fluid will flow from the smaller drop to the larger drop. Passive pumping can be used to collect and load samples in a simple, efficient, and predictable way, particularly on a hydrophobic surface where surface energy is high.

Surface-tension-based fluid flow on the microscale is generally laminar with typical Reynolds numbers below 40 [19-21]. By using a defined volume for the loading sample, the final position and distribution of the sample can be accurately predicted within the electrophoresis device. Similarly, a known volume of fluid can be used to displace a sample of interest out of the microchannel for collection. Previous work has been done to characterize and verify the process of fluid displacement using passive pumping [20] and is represented by Eq. (1). If a channel contains a fluid A, and another fluid B is added to the channel, C indicates the ratio of A to B in the channel after fluid flow has ceased. C is linearly related to the ratio of the channel volume, V_c , to the input volume, V_i . As the volume ratio tends to zero, the channel becomes completely occupied by input fluid, represented by $C = 0$. The value ζ can be determined analytically or experimentally. Theoretically, ζ ranges between 0.33 for a square duct and 0.17 for an infinitely wide duct.

$$C = \zeta \frac{V_c}{V_i} \quad (1)$$

Table 2 gives a list of values for ζ based on ratio of height to width in a rectangular channel. More detailed information on the derivation of ζ can be found here [20].

Figure 1 illustrates the passive pumping process. The sample is loaded into port 1 and flows towards port 2. The portion of the sample that will undergo electrophoresis resides in the separation channel and contributes to the signal strength of the readout. Using Eq. (2):

$$V_{\text{delivered}} = V_i - V_c (1 - C) \quad (2)$$

The amount of sample successfully loaded ($V_{\text{delivered}}$) into the separation channel can be calculated. Based on results in the literature [20], we determined a value of $\zeta = 0.28$ for our channel geometry (Figs. 2 and 3). Our channel volume leading up to the separation channel is

0.74 μL (V_c). For example, by loading a total sample volume of 1 μL , ($C = 0.207$), 0.41 μL is delivered to the separation channel.

During collection, a fixed volume is used to displace the target sample from the separation channel. The fluid flow profiles of sample collection are more complex than in the case of sample loading. The flow profile spreads in the region of the separation channel and cannot be treated as a rectangular duct (channel). However, because the method uses a fixed volume of fluid, the process of sample collection is very simple and repeatable. The fluid at the intersection is displaced towards port 2 in Fig. 2. The separated DNA will then reside at port 2. The sample can then be collected with a pipette or concentrated at port 2 using evaporation.

3 Materials and methods

3.1 Device overview

A schematic for the micro-capillary electrophoresis device is shown in Fig. 3. A straight channel (0.5 mm wide by 47 mm long) is intersected with two orthogonal channels, an injection channel and a collection channel. The channel height is 120 μm . Two reservoirs with planar electrodes are at each end of the channel. Every access port on the device has a hydrophobic patch with a hole in the center made of fluorinated ethylene propylene (FEP, gray boxes) to provide a hydrophobic surface around the port. This provides an optimum surface to control the passive pumping direction. The injection channel (0.25 mm wide by 6.6 mm long) forms a T-junction for sample loading. The collection channel crosses the separation channel. Actual separation distance for the channel from injection to collection is 40 mm. One end of the collection channel has a large collection drop, while the other end has no drop. To initiate pumping a small drop of fluid is placed in the pumping port. The injection portion of the device is filled with $1 \times \text{TBE}$ electrophoresis buffer. The rest of the device is filled with 2.5% HEC in $1 \times \text{TBE}$. The device was constructed using liquid phase photopolymerization to form microchannel structures. Exact details on the methodology used to construct the microelectrophoresis devices can be found in previous work [18].

3.2 Fabrication

Figure 4 shows the fabrication process for the micro-capillary electrophoresis device. Total approximate costs *per* device are \$15 with start-up costs of \$800.00 in materials (*e.g.* transparency masks, poly-isobornyl acrylate (PIBA) polymer, photo initiator, acrylamide coating). A standard spot-curing UV source found in many labs can be used to polymerize PIBA. The micro-capillary electrophoresis device was fabricated with glass and PIBA. Inexpensive gold test slides (2" by 3", \$10.00 each) were purchased (EMF, Ithaca, NY). Each slide had 50 angstroms of titanium followed by 1000 angstroms of gold. Polyimide tape (DuPont Kapton) was cut into 1 mm wide by 20 mm long strips and placed at two opposite ends of the glass slide (length-wise) for electrode patterning. Polyimide tape is resistant to gold etchant and works well for quick patterning of electrodes without the need for traditional photolithography. Electrode positioning was determined by utilizing the transparency mask (cost \$10.00) for channel patterning as a template. The slide was placed in gold etchant (4 g: 2 g:10 mL of $\text{KI:I}_2:\text{H}_2\text{O}$) for 30 s. The slide was then rinsed in diH_2O water for 5 min. The titanium layer was removed by dipping the slide in dilute HF (1:100 in diH_2O) for 20 s, followed by another 5 min rinse in diH_2O . The slide was dried with nitrogen air and the polyimide tape was removed.

The patterned glass slide was used as the device bottom. An adhesive gasket material 120 μm thick (adhesive sheet SA-S-1L, Grace Biolabs, Bend, OR) was cut into 3 mm strips and placed along the length of the glass slide (Fig. 4B). A no. 1 cover-slip (48 mm $\times$ 65 mm Gold Seal cover glass 3335) 150 μm thick and with drilled holes was placed over the adhesive gasket

material creating a seal (Fig. 4C). This formed a glass chamber. Holes were drilled using 1.2 mm diamond tip drill bits (DIB 551.25 from Shor International, Mt. Vernon, NY), and drilled using a Dremel™ drill press at 1000 rpm. To minimize breakage during drilling, 2" by 3", 1 mm thick glass slides were used under the cover-slip glass to provide a flat surface. A drop of water was used over each hole that was drilled and gentle pressure was applied. Because of the thinness of the cover-slip, the time needed to drill 6–8 holes *per* slide was approximately 5 min.

A photo-definable polymer called PIBA was used to form the channels in the capillary electrophoresis chip. Details on the patterning methodology can be found in Beebe *et al* [22]. The PIBA was allowed to flow into the glass chamber. A film mask with a channel pattern on it was placed over the glass chamber (Fig. 4D). The pattern covered the holes on the cover-slip. The chamber was then exposed to UV light for approximately 10 s (7 mW/cm² intensity). Areas of the mask that blocked light were not polymerized while areas that did not block light cross-linked forming a hard brittle material. The unpolymerized material was then removed from the device through the drilled ports *via* vacuum pump using a suction interface (Integrated Dispensing Solutions part no. 8880264). Since the device had a glass surface, the area around the ports had a low contact angle that slowed passive pumping. To increase the contact angle around the port, a patch of FEP adhesive (part 5805T11 McMaster–Carr) was placed over each port to provide a hydrophobic surface. The hydrophobic surface gave a higher contact angle, needed for passive pumping to be stronger and more reliable. Before application to the device the FEP patches were cut into small squares and cored out using an 18-gauge blunt needle (Jensen Global part JG18-1.5, Howard Electronic Instruments, El Dorado, Kansas) that was sharpened with a file going along the edges. The needle was used to core a hole through the FEP adhesive patch allowing access to the port. Figure 4E shows a final device with FEP patches at each port. For imaging purposes dyed 2.5% HEC and buffer were used to fill the device.

To the eliminate electroosmotic flow, the channels were coated with linear acrylamide before performing a separation. This was done by first soaking the microchannel with 0.1 M NaOH for 10 min. The NaOH was removed from the channel using the vacuum pump and rinsed with 5 mL diH₂O. A solution for silanizing the inside glass surface was mixed from 1.5 µL of Bind Silane (Amersham Biosciences 17-1330-01), 700 µL of 90% ethanol, and 160 µL of 10% acetic acid. The microchannel was soaked in this solution for 5 min. This solution was then sucked out and a degassed 3.5% linear acrylamide solution containing 1 µL of TEMED and 1 mg of potassium persulfate *per* milliliter of solution is sucked into the microchannel and allowed to remain for 45 min. Any acrylamide that did not attach to the glass surface was sucked out, and the channel was rinsed with 5 mL of diH₂O.

3.3 Biological materials

The sieving buffer used in all experiments was 2.5% HEC (Fluka Biochemical 54290) dissolved in 1 × TBE buffer (pH 8.3, Fisher Scientific). The buffer was filtered through a 0.45 µm filter before use. Promega BenchTop 100 bp ladder (part G829A) was used for dsDNA separations. Oligonucleotides labeled with 5-carboxyfluorescein (5-FAM) and of 91 and 118 bp length were ordered from the University of Wisconsin Biotechnology Center. For experiments using unlabeled dsDNA, SYBR Safe DNA stain (Invitrogen S33102 10 000 × concentrate) was added to the sieving buffer at a ratio 1/100. For experiments using ssDNA (oligonucleotides) samples were mixed with Promega Blue Dextran Loading Solution (catalog DV4351, composition 88.25% formamide, 15 mg/mL blue dextran, 4.1 mM EDTA) at a ratio of 1:1. The ssDNA samples were then heated to 96° for 5 min and cooled on ice before injection into the device. For all separation experiments 0.5 µL drop of DNA was placed at the injection port using a micropipette.

3.4 Device operation

Sieving buffer was loaded into the device using a vacuum pump by placing small drops at each of the access ports the vacuum pump did not occupy. Once the channel was completely filled with 2.5% HEC, the pump was taken to the injection port and buffer was removed from the injection region of the micro-capillary electrophoresis chip shown in Fig. 4E. Drops of $1 \times$ TBE were placed at the electrode reservoir closest to the injection port and sucked into the device forming a liquid interface between the HEC buffer and TBE. The viscosity of 2.5% HEC is relatively high compared with TBE. This allowed us to fill the injection region of the electrophoresis chip before HEC buffer was removed from the rest of the device. TBE was used in the injection region for two reasons. First, it aids in stacking samples. The mobility of DNA is much higher in TBE than it is for 2.5% HEC because TBE has low viscosity. This allows the sample to stack at the interface between the two buffers. Second, because of this lower viscosity, passive pumping is faster in TBE resulting in quicker injection times.

After buffers were loaded into the device a 5 μ L drop of buffer was placed over each electrode. This served to maintain electrical conductivity and prevent bubbles produced at the electrodes during electrophoresis from getting into the separation channel. Any bubbles formed at the electrode float to the top of the drop keeping it from the microchannel network. Bubbles are formed due to the hydrolysis of the separation buffer that results in the formation of hydrogen or oxygen gas. Bubbles in the separation channel can reduce the uniformity the electric field, which can distort bands during electrophoresis. The electric field applied was 87 V/cm (Thermo EC 6000P electrophoresis power supply). All imaging was done using a Leica DFC300FX camera on an Olympus SZX12 stereoscope with an Olympus UV light source (model BH2-RFL-T3). CamStudio video capturing software was used to take video of the separations and ImageJ was used to extract frames from the video and analyze fluorescent intensities. DNA quantification was done using a Biotek Synergy plate reader.

4 Results and discussion

4.1 Sample injection

Figure 5 shows a 91 bp oligonucleotide sample being injected into the micro-capillary electrophoresis device. The injection region of the device is filled with TBE buffer. A large TBE drop of 25 μ L is placed at the port over the electrode (Fig. 3). An injection drop (containing the sample) of 0.5 μ L is used to pump the sample towards the T-junction (Fig. 5A). The sample begins to fill the separation channel (Fig. 5B) with the majority of it flowing towards the collection drop (Fig. 5C). Once the injection drop is consumed, an electric field is applied (Fig. 5D) and the sample begins to move down the separation channel (Fig. 5E). Any DNA remaining in the injection channel after the electric field is applied eventually recedes from the edge of the separation channel. Figure 5F shows a band beginning to form. Total time for sample injection is 70 s. If the sample is injected and left to sit for more than 30 min, the plug can diffuse towards the negative electrode, which can effect the initial stacking of the DNA through the separation medium.

4.2 Sample separation

To demonstrate that separation of DNA is not adversely affected using passive pumping injection, an off-the-shelf 100 bp ladder was separated using this injection technique. The Promega BenchTop 100 bp ladder consists of 11 dsDNA fragments with sizes 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, and 1500 bp. The 500 bp band is present at a higher intensity than the other bands to serve as a reference indicator. Figure 6 shows separation of the DNA ladder measured using fluorescent intensity *versus* time of detection. The peaks shown fit well with the reference image provided by the manufacturer with 500 bp showing the highest intensity as expected. The total separation time for this sample was approximately 160 s. This

separation can be done much faster by increasing the electric field strength. However, since we were monitoring the separation and injection in real time, lower field strengths were used to observe sample behavior.

4.3 Sample extraction

Sample collection from the micro-capillary electrophoresis chip is shown in Fig. 7. The side walls of the channels are shown with dashed lines. A 91 bp band moves along the separation channel (Fig. 7A). A 25 μL collection drop of DNase/RNase free water is placed at one end of the collection channel. As the band reaches the collection channel/intersection, it begins to spread out as it is no longer confined within the 0.5 mm wide separation channel (Fig. 7B). When the band reaches the middle of the intersection point of the collection channel and the separation channel, the electric field is stopped (Fig. 7C). A pumping drop of 5 μL DNase/RNase free water is used to pump the band towards the collection drop (Fig. 7D). Pumping time is approximately 60 s. The sample moves through the collection channel (Fig. 7E) and to the collection drop (Fig. 7F). Several pumping drops ($10 \times$) of 5 μL are used to completely wash out the DNA from the collection channel, thus total wash time was 5 min. A pumping drop volume of 5 μL was used for these experiments; however larger volumes can be used for pumping as long as the volume is smaller than the collection drop. If larger drops are used then the pumping speed is decreased. Characterization of pumping velocity due to drop size has been published by Berthier *et al.* [21]. Although not shown in this work, the collection time (pumping speed) can be increased by keeping TBE in the collection region as it is kept in the injection region. The lower viscosity of TBE allows the DNA to be collected faster than from the viscous HEC buffer.

When DNA is processed using traditional methods, a minimum amount of DNA is needed for successful analysis. To assess the amount of DNA recovered from the device using this technique a known amount of DNA was injected into the device and the amount recovered was measured using fluorescence and a plate reader (Biotek HT Synergy). A calibration curve was created using FAM-labeled DNA (in 100 μL drops) at concentrations of 4, 1, 0.5, 0.2, 0.125, 0.0625, 0.03125, and 0.015625 μM , to relate the fluorescent intensity to the extracted DNA concentration (see Supporting Information). The number of moles and molecular weight of the purified oligonucleotides is given by the manufacturer (University of Wisconsin Biotech Center). Using this information the amount of DNA (in ng) can be calculated for input and output samples. Equation (2) was used to calculate the amount of DNA successfully loaded into the separation channel. Devices with 0.5 mm wide separation channels and 1 mm wide separation channels were used. The 1 mm wide channels were used to increase the amount of volume injected and thereby increase the amount of DNA to be recovered from the device. For the device with 0.5 mm wide separation channels, a 0.62 μL sample of 91 bp ssDNA at a concentration of 5 μM was injected into the separation channel. For the device with 1 mm wide separation width channels, a 0.8 μL sample of 118 bp ssDNA at a concentration of 15 μM was injected into the separation channel. Table 3 shows initial results of two experiments.

As seen from Table 3 the amount of DNA recovered can vary. This is due to the positioning of the band in the collection channel (Fig. 7C). If a band is not placed optimally in the pumping path (either by allowing it migrate too far or not far enough), the amount of sample recovered can decrease greatly. We found recoveries as low as 48% when the band was not stopped at the intersection of the collection channel and separation channel. Appropriate positioning of the band is achievable by slowing the band migration rate (1 cm/min) down before it reaches the intersection. This allows the user to visually ascertain the position of the band and shut off the electric field by pushing a button. Migration is stopped instantaneously as the power supply is stopped. However, the amount of DNA recovered is still sufficient enough for performing traditional molecular biology techniques such as PCR.

After the band moves from the separation channel through the collection channel, some DNA can remain at the channel wall where the fluid velocity goes to zero due to the no-slip boundary condition. With time, the DNA along the channel wall can diffuse out into the center of the channel and subsequently be washed away into the collection drop. However, diffusion times for DNA can be quite long (500 s to move 100 μm with a diffusion coefficient = $10^{-5} \text{ cm}^2/\text{s}$ in PBS [23]) and we again rely on Eq. (1). In this case, C indicates the ratio of biological material to fluid in the channel after flow has ceased. For complete washing, a value of 0 is desired for $C = \zeta(V_c/V_i)$. Therefore, the washing volume, V_i , must be several times larger than the volume of the collection channel, V_c . More details on channel washing using passive pumping have been described by Warrick *et al.* [20]. Two important assumptions made in the derivation of Eq. (1) are that the channel cross-section is uniform and fluid viscosities are matched. As mentioned earlier, the intersection of the extraction channel and separation channel complicates flow profiles. Therefore, in this case, the equation applies only to material that has been displaced into the extraction channel after the first pulse of fluid. Despite this, an appropriate value for V_c/V_i that will indicate nearly complete removal of residual DNA in the extraction portion of the channel can be estimated empirically.

Figure 8 shows how residual DNA in the collection channel can be washed out by passive pumping. Initially, the DNA band is pumped towards the collection drop (with a 5 μL pumping drop) leaving a trail of sample along the channel wall (Fig. 8A). After 15 μL of washing, more of the DNA sample is removed, and eventually the majority of it is removed after 45 μL of washing (Figs. 8B–D). Figure 8B shows the normalized intensity taken over the channel for Figs. 8A–D. The fluorescent intensity inside the channel without DNA was first measured and compared with various stages of washing. Then DNA was pumped/washed through the collection channel using pumping drop increments of 5 μL . The intensity reading was taken using ImageJ along the length of the channel shown in Fig. 8E. The collection channel dimensions for this device were 1 mm wide by 4.36 mm long. Calculated C values are listed next to each bar. Figure 8E shows that after 45 μL of washing, the intensity within the channel matches that measured for a channel without DNA. While it is difficult to completely wash out the collection channel, C of 0.003 shows no fluorescence of DNA indicating a channel with little residual DNA. Thus, using pumping/washing volumes that give a C value of 0.003 should minimize the amount of residual DNA left in the collection channel. Channel washing is very important if more than one band is going to be extracted from the micro-capillary electrophoresis chip or if the band of interest is not the first band to reach the collection channel. When bands are not extracted and allowed to cross the collection channel, they will leak into collection channel. This has been observed in previously published work [24] and researchers have approached this problem by using addressable electric fields within microelectrophoresis channel networks [24,25]. This involves using shaped electric fields to improve extraction of DNA by reducing band expansion and carry-over into the collection channel when unwanted DNA bands pass by. Although successful, this method requires more complex electrode operation as localized fields are placed in the microchannel using extra electrodes. In the case of our device we simply wash this unwanted DNA out of the channel using passive pumping minimizing the effect on the band of interest. The procedure requires that the electric field be shut off so that the bands stop moving. Washing through the channel can be accomplished in a few minutes so that any diffusing of bands to be collected will be minimal. Once the electric field is turned on, the bands will condense again. A second alternative to this approach would be to collect every band that reaches the collection channel. In addition, when bands are collected, a small portion of the band goes back into the separation channel as depicted in Fig. 2c. This portion can be collected by driving it back (*via* electrophoresis) into the intersection or discarded by driving it all the way to the positive electrode. The intersection can then be washed *via* passive pumping as described above for further collection of bands.

5 Concluding remarks

The work presented here illustrates an inexpensive micro-capillary electrophoresis device that uses passive pumping for sample injection and extraction. This injection methodology is predictable and reliable and showed no adverse affects on the DNA separations presented here. Most life science researchers have not taken advantage of microscale electrophoresis because of the high cost involved in making or purchasing the chip, or because operation of such devices requires expensive, specialized liquid handling equipment not already present in their lab. The device presented here removes these barriers. First, the fabrication of the device can be done using liquid phase photopolymerization technology without the need for expensive microfabrication equipment traditionally associated with microelectrophoresis chips. Second, the operation of the device is done with micropipettes and electrophoretic power supplies already present in most life science labs. No specialized macro- to microscale interface is needed. The detection of DNA can be done with a UV source, camera, and microscope commonly found in most labs. The device presented here showed the use of a single channel for DNA separation. For separations that require a DNA ladder for reference, parallel channels can be fabricated to allow simultaneous separations. Alternatively, a standard can be introduced into sample that is being separated as it is done with commercial capillary electrophoresis systems and sizing can be interpolated by looking at the relationship between the standards and the unknown DNA size [26].

This accessible micro-capillary electrophoresis device also makes sample collection off-chip possible, making it a viable alternative to traditional gel techniques where DNA bands are physically cut and removed from the gel. The passive pumping extraction method presented here is simple to use and also has the added benefit of increasing the volume to a manageable (5–50 μ L) size. Sample extraction from capillary electrophoresis machines and chips is not commonly done leaving researchers to only use gels if they need to process a sample after separation. This added utility of sample extraction can make this device more attractive for these applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

FEP	fluorinated ethylene propylene
HEC	hydroxyl ethyl cellulose
PIBA	poly-isobornyl acrylate

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Figure 1.

Sample loading of microelectrophoresis device using passive pumping. Before pumping, the microelectrophoresis chip is full of separation buffer (Fluid A). (A) A volume (V_i) of DNA (Fluid B) is loaded into port 1 and flows towards port 2. V_c is the volume of the channel and port leading to the separation channel. Equation (1) defines the value C as the ratio of Fluid A to Fluid B after flow has ceased. This ratio is then used in Eq. (2) to calculate the amount of sample delivered ($V_{\text{delivered}}$). (B) An electric field is applied driving the DNA down the separation channel.

Figure 2.

Sample collection in a device using passive pumping. (A) A band migrates down the separation channel until it intersects with the collection channel. (B) A large drop of buffer is placed at port 2. A micropipette delivers a fixed volume smaller than the volume at port 2 to port 1. The pressure from the small drop begins to displace the band at the intersection. (C) A fixed volume of fluid is used repeatedly to displace the separated band into port 2 for aspiration or concentration.

Figure 3.

A schematic for the micro-capillary electrophoresis. A straight channel is intersected with two orthogonal channels, an injection channel, and a collection channel. Each channel depth is 120 μm . Two reservoirs with planar electrodes are at each end of the channel. Every access port on the device has a hydrophobic patch with a hole in the center made of FEP (white boxes) to provide a hydrophobic surface around the port. This provides an optimum surface to control passive pumping. The injection channel forms a T-junction for sample loading. The collection channel crosses the separation channel. Actual separation distance for the channel from injection to collection is 45 mm. The top end of the collection channel has a large collection drop, while the other end has no drop. To initiate pumping a small drop of fluid is placed at the pumping port. The injection portion of the device is filled with $1 \times \text{TBE}$ electrophoresis buffer. The rest of the device is filled with 2.5% HEC in $1 \times \text{TBE}$.

Figure 4.

Fabrication materials and process for micro-capillary electrophoresis chip: (A) FEP adhesive to provide a hydrophobic surface around ports; (B) pattern gold electrodes and placing adhesive spacers on gold slide; (C) the cover-slip is placed on the adhesive spacers. The holes are aligned over the electrodes forming a chamber. (D) The glass chamber is filled with IBA, a mask is aligned to the holes and then exposed to UV light. The areas blocked by the mask from the channel. The polymer that does not cross-link is removed from the device by a vacuum pump with a suction cup. (E) The cut FEP pieces (outlined by dashed boxes) are placed over each port to provide a hydrophobic surface for passive pumping. The separation channel is filled with HEC and the injection channel is filled with buffer (dyed for imaging purposes). A DNA sample is injected into the buffer filled channel *via* the injection port. The DNA is then directed to the HEC-filled channel for separation.

Figure 5.

Injection of 91 bp ssDNA sample into a micro-capillary electrophoresis device by passive pumping. (A) T-junction before sample arrives. (B) Sample begins to fill the separation channel. (C) Sample is pumped towards the right where a large collection drop (25 μL) of TBE buffer is placed. A sample pumping drop of 0.5 μL was used. (D) An electric field is applied and the sample leaves the injection channel. (E) The samples begins to stack and move down the separation channel. (F) A band starts forming. Total injection time is approximately 70 s.

Figure 6. Separation of Promega Bench-Top 100 bp ladder in micro-capillary electrophoresis chip using passive pumping injection. As expected, 11 peaks were detected with a higher intensity reading for the 500 bp DNA sample.

Figure 7.

Sample extraction of 91 bp ssDNA from the micro-capillary electrophoresis device by passive pumping. Channel walls outlined with dashed line. (A) A band travels through the separation channel towards the extraction intersection. (B) The band reaches the intersection and begins to spread. (C) Once the band reaches the midpoint of the collection channel, the field is stopped. (D) A large collection drop (15 μ L) of DNase/Rnase-free water is placed at one end of the collection channel and a smaller pumping drop (5 μ L) is placed at the other end. This begins to drive the sample towards the collection drop (upwards in this case). After the pumping drop is consumed another one is added to pump again. This is done to ensure the maximum amount of DNA is extracted from the chip and is repeated up to ten times. (E) The sample moves through the collection channel. (F) Sample is collected at the collection drop, which is then removed off chip using a micropipette.

Figure 8.

Recovery of 118 bp ssDNA from collection channel (1 mm wide by 8.72 mm long). A large volume of DNase/Rnase-free water is used to pump the DNA band through the collection channel. (A) Initially a 5 μL drop is used to pump the band through the collection channel. Some DNA is left at the channel wall due to the no-slip boundary condition. (B–D) Additional water is added until the DNA is washed away from the collection channel. (E) Graph of normalized intensity readings of channels shown in (A–D). Initially a reading was taken for the channel without DNA and compared with fluorescent readings during channel washing. C indicates the volume displaced in the collection channel. A lower value indicates more complete displacement. (a) DNA in collection channel after initial 5 μL pumping drop/wash. (b) DNA in collection channel after 15 μL wash. (c) DNA in collection channel after 25 μL wash. (d) DNA in collection channel after 45 μL wash.

Table 1

Sample injection methods for microelectrophoresis

Injection method	Advantages	Disadvantages
Electrokinetic	Robust, precise plugs, fast injection	pH and ionic strength of the buffer requires careful adjustment for consistent injection or sample biasing can occur [16,17]. Complex setup (powers supplies, tubing)
Pressure/pneumatic	Precise injection plugs, not susceptible to sample biasing	Complex setup, tubing, syringe pumps, valves needed for injection
Passive pumping injection	Simple setup, no tubing or valves required for sample delivery. Same method can be used for sample extraction	No dynamic change of injection rate as the rate of injection is dictated by channel geometry, and precise drop size manipulation

Table 2

ζ values for different aspect ratios in a rectangular microchannel

Height/ width	1	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0
ζ	0.31	0.31	0.30	0.30	0.29	0.28	0.27	0.26	0.24	0.21	0.17

Table 3

Recovery of DNA from microelectrophoresis chip

DNA injected	DNA recovered (ng)	% Recovered
360.3 ^{a)}	278.2	77.2
51.5 ^{b)}	42.7	84.9

^{a)}Extraction from 1 mm separation width channel.^{b)}Extraction from 0.5 mm separation width channel.