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Short communication

An injection molded microchip for nucleic acid purification from 25 microliter samples using isotachophoresis

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ABSTRACT

We present a novel microchip device for purification of nucleic acids from 25 μL biological samples using isotachophoresis (ITP). The device design incorporates a custom capillary barrier structure to facilitate robust sample loading. The chip uses a 2 mm channel width and 0.15 mm depth to reduce processing time, mitigate Joule heating, and achieve high extraction efficiency. To reduce pH changes in the device due to electrolysis, we incorporated a buffering reservoir physically separated from the sample output reservoir. To reduce dispersion of the ITP-focused zone, we used optimized turn geometries. The chip was fabricated by injection molding PMMA and COC plastics through a commercial microfluidic foundry. The extraction efficiency of nucleic acids from the device was measured using fluorescent quantification, and an average recovery efficiency of 81% was achieved for nucleic acid masses between 250 pg and 250 ng. The devices were also used to purify DNA from whole blood, and the extracted DNA was amplified using qPCR to show the PCR compatibility of the purified sample.

1. Introduction

Isotachophoresis (ITP) for nucleic acid purification is a sample preparation technique with a growing portfolio of applications. ITP has been used to isolate DNA from human blood [1–3], serum, and plasma [4–7], as well as RNA purification from bacteria in urine [8] and blood [9], and purification of small RNA from total RNA from kidney cells [10]. ITP purification is a valuable alternative to conventional solid-phase extraction and liquid–liquid extraction techniques for nucleic acid purification as it can extract from very low abundance samples and does not require centrifugation, buffer exchanges, pumping, or toxic chemistries.

To date, the total extraction efficiency and sample preparation volumes addressed by on-chip ITP purification have been limited by the small dimensions associated with off-the-shelf electrokinetic chips. Typically, the work has been performed in etched glass microchannels, with injection volumes of order 100 nL [3,10]. Separation in larger plastic microchips with channel volumes on the order of 3 μL was demonstrated, but the injection methods used in these devices still limited extraction efficiencies to order 1% [2]. Here, we demonstrate an injection-molded plastic microfluidic chip for ITP purification of nucleic acids that achieves high extraction efficiencies and is capable of processing 25 μL of blood lysate in a single experiment. We describe the design features of this chip, and analyze its performance using fluorescence quantification and quantitative PCR (qPCR).

The higher recovery efficiency demonstrated here is primarily due to two factors: A separation channel volume which is on the order of the sample volume, and the use of dedicated electrolysis reservoirs free of sample. The separation channel is 2 mm × 0.15 mm × 100 mm. The 0.15 mm depth achieves sufficient heat rejection while the 2 mm width achieves rapid processing. We placed electrodes in dedicated buffering reservoirs, which are free of sample and contain high local buffer concentration. These reservoirs are effective in buffering pH insults caused by electrolysis, even when large volumes are processed. The design draws on scaling analyses for ITP purification as presented by Marshall et al. [11].

2. Materials and methods

2.1. Device design and features

We generated the geometry of our custom microchip using commercial computer aided drawing (CAD) software (AutoCAD, AutoDesk, San Rafael, CA). The design has a nominal channel depth of 0.15 mm, a nominal width of 2 mm, and a total channel length (sum of sample and separation sections) of 200 mm. The channel is

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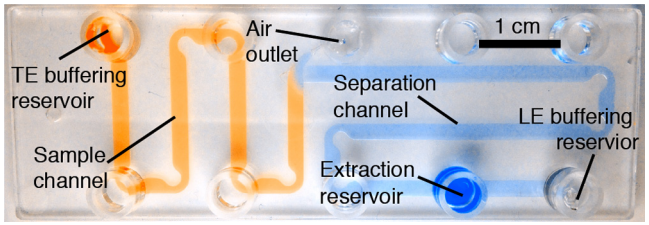

Fig. 1. Image of the finished device, here with channels loaded with water dyed with blue and red food coloring. The sample channel (on the left half of the 25.5 by 75.5 mm chip substrate) appears orange, while the separation channel is blue. A more detailed view of the junction between these two channels and the structure of the connected air outlet channel is shown in Fig. 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

divided into a sample channel, with a total volume of 25 μL, and a separation channel, with a total volume of 30 μL. The design includes four reservoirs with access to the fluidic channel, and each reservoir can hold a total volume of 70 μL. Each reservoir is designed to be compatible with Leur lock connectors. The CAD files for the finished device are available in the supporting information (SI), and described in SI-1.

Our microfluidic device design was fabricated by a commercial microfluidic foundry (Microfluidic Chipshop Gmbh, Jena, Germany). The devices were fabricated by injection molding of the fluidic layer and reservoirs in a single monolithic piece. These devices were manufactured both in poly-methyl methacrylate (PMMA) and Topas, a cyclic olefin copolymer (COC). The devices were sealed with plastic films with thickness of 175 μm (PMMA) or 140 μm (COC), respectively. The finished device is shown in Fig. 1.

Each channel corner in the device is an optimized 90° turn designed by Molho et al. to minimize electrokinetic dispersion due non-uniform electric fields in the turn [12]. These turns have a constriction ratio of 0.5 and a recovery ratio of 1.0, as defined by Molho. Despite the self-sharpening nature of ITP, we found that dispersion added to ITP zones by standard turns can persist for order 10 channel widths and longer. We found that the application of low-dispersion turns reduced this self-correction of ITP to just a few channel widths. Slow self-correction seems to be especially a challenge with low diffusivity analytes like genomic DNA.

The reservoirs in the device are arranged so that the two electrode-containing reservoirs can be configured for extremely high buffering capacity without affecting the chemistry of either the sample or the extracted nucleic acids [13,14].

We designed and introduced specialized loading structures at the junction between the sample and separation channels to allow the formation of a sharp interface between the sample solution and the leading electrolyte buffer. These structures also enable loading of the sample into the device without wasting liquid into the vacuum port. These structures operate in a manner similar to the phase-guide described by Vulto et al. [15,16], or the more commonly applied microfluidic capillary valve structures [17–19]. We use flat-surface ramps that reduce the channel height from 150 μm to 75 μm leading to and terminating at a sudden expansion in channel height back to 150 μm. This ramp and sudden expansion create what we term capillary barriers. Liquid wicking up the ramp and up to the "ledge" structure formed by the sudden expansion faces an energetic barrier associated with flowing into the expansion (as additional liquid surface area is required for the liquid to advance). These valve structures are oriented so that the liquid arrested by the capillary barrier can be wetted by liquid from an adjacent channel, creating a bubble-free liquid-to-liquid interface. The vacuum-actuated filling and liquid-to-liquid mating operation enabled by these structures are summarized in Fig. 2.

Loading was tested in both the PMMA chips and the COC chips. Both materials exhibited approximately the same performance, with the exception of the filling step. In the COC devices, liquid does not spontaneously wick into the channel, and vacuum was used to induce filling, as shown in Fig. 2. In the PMMA chips, fluid

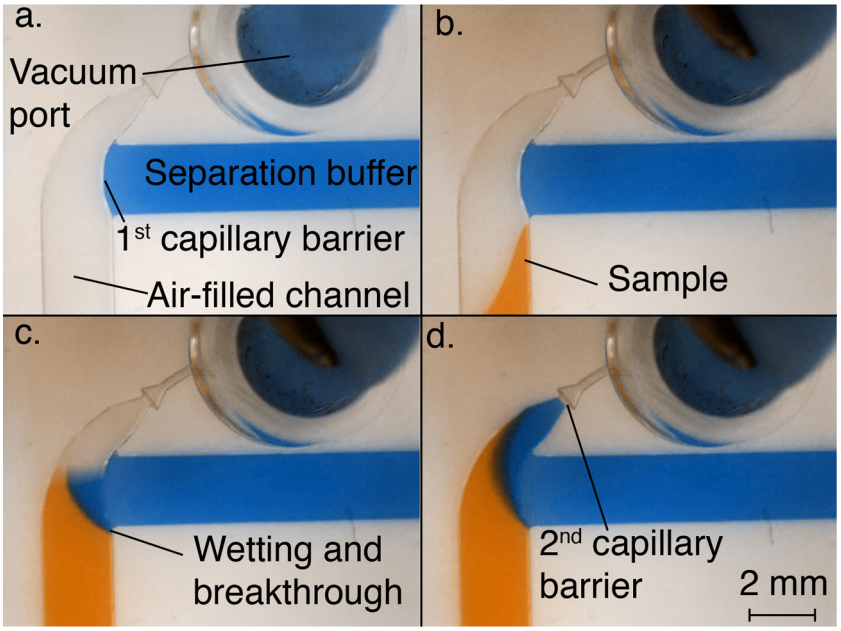

Fig. 2. Stages in capillary-barrier-aided loading of the COC chips visualized using food coloring in water. (a) The blue liquid, simulating separation buffer, is initially loaded into the separation channel. −0.1 psig vacuum is applied at the vacuum port, and the separation buffer flows to the capillary barrier, where it stops at the precise edge of the expansion downstream of the ram. (b) The red liquid, simulating the sample solution, enters through the sample channel. (c) The sample solution forms a liquid-to-liquid interface (repeatedly free of bubbles) with the separation buffer. The two solutions then flow in parallel toward the air outlet. (d) The volume near the liquid-to-liquid interface is run up against and stopped by a second capillary barrier inside the vacuum port. This avoids waste of either liquid into the vacuum port, and keeps the vacuum connection dry. A sharp interface is preserved between the two liquid zones and the ITP process is ready to begin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)