ABSTRACT
Here we report the development of a fully integrated microfluidic chromatography system: A novel column geometry solves a long-standing problem in on-chip separations. This geometry facilitates the rapid and high yield stacking of multiple bead columns in poly(dimethylsiloxane) PDMS microfluidic devices using low pressure flow. Microcolumns are integrated with on-chip plumbing to enable fully automated sample loading, programmable gradient generation, gradient storage, separation, and fluorescent detection. We demonstrate this system in the high resolution separation of fluorescently labelled DNA using a strong anion exchange (SAX) resin.

KEYWORDS: DNA Separation, Integrated Liquid Chromatography, Microcolumn

INTRODUCTION
Microfluidic systems offer advantages of small volume processing, excellent reproducibility, and automation, making them attractive platforms for the integration of analytical and preparative sample separation methods. Although separation by electrophoresis on microfluidic devices has been studied extensively [1], these applications are generally analytical and are not well-suited to recovery of purified products. By comparison, the use of solid-phase chromatography is much less developed, largely due to difficulties in reliably stacking columns with an immobile phase. Robust integration of microsystems for liquid chromatography remains of high interest and has the potential to open many new applications since the mode of chromatography can be changed as needed by selection of the appropriate resin.

Stacking of microchannels with chromatography beads requires the preparation of a packing material suspended in an appropriate solvent and introducing it into a column under pressure. Due to the large impedance of bead columns, conventional stacking techniques require pressures of up to 5.5MPa (800psi) in order to produce high-quality columns. Due to the high required backpressures, the use of integrated chromatography columns in microsystems has been restricted to devices made from hard materials such as glass or silicon [2] that are not easily amenable to microvalve integration. For this reason both the level of integration and parallelization of on-chip chromatography systems has been limited. PDMS devices made using Multilayer Soft Lithography (MSL) allow for dense integration of monolithic valves [3,4], but are practically limited to pressures below 340kPa (50psi) due to failure of layer-layer adhesion.

To enable the use of integrated valves with liquid chromatography, we have developed a column geometry that allows for the parallel low-pressure stacking of high-quality columns in PDMS devices within minutes and with exceptional reproducibility. The combination of this column technology with MSL-based valving was used to implement a completely
integrated chromatography system for DNA separation. Non-porous SAX resins are well-suited for on-chip separation of DNA at physiological pH: the negatively charged DNA backbone interacts with positively charged quaternary ammonium functional groups on the SAX resin. The overall negative charge of each DNA fragment is proportional to its length, thereby allowing for elution of progressively longer fragments through application of an increasing salt gradient.

The novelty of the reported system is threefold: firstly, we show that our microfabricated column geometry allows, for the first time, the stacking of arrays of high-quality chromatography columns up to several centimetres in length with high yield; secondly, the ability to handle sub-nanolitre volumes on-chip coupled with small diameter columns enables preparative separation of nucleic acids with low dilution and high detection sensitivity [5,6]; and thirdly, we use microvalve technology to achieve the first fully integrated PDMS liquid chromatography system on chip, with all elements required for chromatographic separation.

EXPERIMENTAL

A fully integrated microdevice for liquid chromatography is shown in Figure 1. Due to the relatively low flow impedance during the stacking process, multiple 20mm long columns can be stacked simultaneously within less than ten minutes at a pressure of 200kPa (30psi). The array of chromatography columns is connected to a chip internal mixer which keeps the immobile phase, SAX beads (5µm, non-porous SAX, Sepax Proteomix), in suspension and allows for a rapid injection of the resin into the chromatography columns.

The chromatographic system is complemented by an integrated gradient generator. The gradient is formed by use of valve actuations to gradually increase the relative amount of the buffer that favours sample elution. The gradient generator works by changing the relative flow impedance for each buffer using two identical sets of variable fluidic resistors. The two buffers to be mixed comprise a low salt buffer A (0.1M NaCl, 10% ACN, 25mM Tris (pH 7.5), 1mM EDTA, 0.1% Tween 20, 15mM NaN3) and a high salt buffer B (A+1M NaCl). Valves states for each resistor bank are chosen in a complementary fashion that preserves the total flow rate such that any combination of one resistor network is reversed in the other set.

In order to facilitate fast mixing of the two buffers, a multi-laminate mixer is incorporated between the gradient generator and the gradient storage line. Each of the two inlet channels is split into 25 streams which are interlaced in an alternating fashion using interlayer connections and joined in a single channel [7]. After passing through the multi-laminate mixer, the gradient is directed to a storage channel which holds the entire gradient before it is applied to the chromatography columns. Following column clean-up and equilibration, up to 1ng of sample (FAM labelled ssDNA, 100-2000bp, BioVentures) was loaded per column and separated by applying the previously generated gradient at a flow rate of 150nl/min (25psi backpressure). Chromatography peaks were detected by fluorescent imaging at the column outlet at a wavelength of 510nm.

RESULTS AND DISCUSSION

The flow pattern during the stacking process has been optimized to produce tightly packed columns by adjusting channel dimensions and impedances. This ensures that buried cavities in the chromatographic resin do not occur since the stacking process progresses from the column inlet to the bead inlet.

Reliable chromatographic separation requires the applied gradient to be highly reproducible and well controlled: the resistor network has proven to be ideally suited to produce a wide range of gradient shapes and slopes (Figure 2a). Using a multi-laminate mixer, complete mixing of the two buffers can be achieved in less than 0.1s as splitting the buffer streams into multiple sub-streams reduces the diffusion length to 4µm. A flow rate of 0.5µl/min minimizes the effect of Taylor dispersion during gradient transport and produces concentrations that accurately reflect programmed values.

![Figure 2: a) Gradient measured at the mixer outlet (0-100% buffer B, 10min). Error bars represent standard deviation from the mean (n=4). b) Chromatogram of a FAM labelled DNA ladder (100bp, 200bp, 500bp, 1000bp, 2000bp). Two subsequent linear gradients have been applied: 0.7M to 0.85M in 8min followed by 0.85M to 0.9M in 10min.](image-url)
Figure 2b shows the high resolution separation of DNA fragments using SAX chromatography on chip. Near baseline resolution was achieved by subdividing the gradient into a steeper section for DNA fragments between 100bp and 500bp (0.7M to 0.85M NaCl in 9min) followed by a shallow gradient for 1kb and 2kb fragments (0.85M to 1M NaCl in 30min).

CONCLUSION
The microfluidic integration of high quality chromatographic columns coupled with on-chip gradient generation stands to greatly expand the analytical and preparative capabilities of microfluidic devices. In particular, robust column stacking in PDMS devices allows for the seamless integration of on-chip nanolitre volume sample handling with the flexibility of optimized solid phase separation resins. The ability to perform nucleic acid size selection on sub-nanogram samples of DNA holds promise for on-chip genomics applications including sequencing library preparation, cloning, and sample fractionation for diagnostics.

REFERENCES

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