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Authors
He, Qing
Xie, Jun
Tai, Yu-Chong
et al.

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Qing He, Jun Xie, and Yu-Chong Tai
Electrical Engineering Department, California Institute of Technology,
Pasadena, CA 91125
Yunan Miao and Terry D. Lee
Division of Immunology, Beckman Research Institute of the City-of-Hope
Duarte, CA, 91010

ABSTRACT

This paper presents the first direct delivery of silica beads into separation columns using bead/photoresist mixture for on-chip Liquid Chromatography (LC) – Electrospray Ionization (ESI) applications. Instead of packing beads into an existing column from off-chip, this new method uses photoresist mixed with surface-functionalized LC silica beads for a modified photolithography (including spin-coat, exposure and development) and parylene-channel process, followed by sacrificial photoresist removal (by acetone) to release the silica beads inside the LC column. The substantiation and validation of this new technique is demonstrated on an LC-ESI chip integrated with particle filters, silica-bead LC column, and electrospray nozzle, which has been tested both physically and chemically.

INTRODUCTION

High Performance Liquid Chromatography (HPLC) is one of the most powerful, versatile, and widely-used separation techniques. It allows separation, identification, purification, and quantification of the chemical compounds in complex mixtures. HPLC separation column is usually packed with micron-sized particles with a diameter from 1 to 10 μm. The particles are coated with surface-functional groups (stationary phase) to interact with the sample and eluent (mobile phase). When the injected sample plug is carried through the column by the liquid eluent, different sample components interact with the stationary phase differently, thus partition their time in stationary and mobile phase variously, and therefore migrate through the column at different speeds, and exit the column at different time.

Normally, LC columns are made by externally packing beads into a capillary tube. Although highly desirable, on-chip LC (unlike electrophoresis) is rarely published [**1-7**] partly because the integration of LC columns without external packing remains a difficult challenge. To make columns, packing with on-chip frit [**1**] [**2**] and packing without frit [**3**] have been demonstrated. Alternative methods have also been proposed to avoid packing, such as open-tubular [**4**], coating nanoparticles on channel surface [**5**], coating micromachined posts arrays [**6**], and monolith [**7**]. We have previously demonstrated the on-chip separation and detection of seven-anion mixture with externally packed Ion Liquid Chromatography microchip with integrated frits/filters, injector, conductivity detector, and column externally-packed with ion-exchange LC beads [**1**]. Unfortunately, although it may seem to be straightforward to pack a fabricated channel, the packing process is laborious, time-consuming and one column at a time. Therefore, we propose a new in-process bead-delivering technique to make integrated LC columns. This method is batch manufacturable, can be easily integrated with other devices, and does not depend on bead types.

The combination of liquid chromatography and Mass Spectrometry (LC-MS) is a very powerful merger for separation and detection, where ESI is usually used for coupling. Microchips with LC-ESI capabilities are highly desirable. We have successfully made parylene freestanding ESI nozzles before [**8-9**]. This work for the first time integrates the LC beads column with ESI nozzle, using the above-mentioned beads integration technology.

DESIGN AND FABRICATION

The major steps for integrating beads into a micromachined LC column are shown in Figure 1. First the beads are mixed with photoresist. The mixture is spin-coated and photo-patterned together. Parylene is used to form channels and filters, where filters are channels with height smaller than the bead diameter. After photoresist removal by Acetone, the beads are free to move in the channel but trapped by filters at ends of the channel. Figure 1(d) illustrates a densely packed column with 5μm-diameter LC silica beads with C18 coatings.

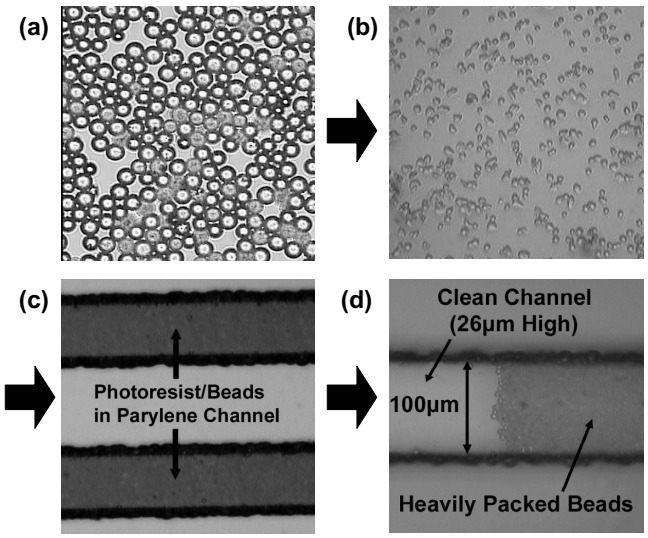

Figure 1. *Pictures illustrating the major steps for integrating beads into parylene channel.*

The photo-patterning process can leave a bumpy surface after developing. This is due to beads either exposed on or removed from the top surface during developing. Therefore, the photo-patterned structure surface roughness is about ± bead diameter. Those beads that are exposed after photo-patterning tend to stick to subsequently deposited parylene and do not release from parylene

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even after photoresist removal (Fig. 2 (a)). To prevent beads sticking to channel-top parylene, a buffer photoresist layer can be spin-coated on top of the mixture film and patterned together with the mixture. The result can be seen in Figure 1 (d) and 5 (d) where essentially no bead stuck to top parylene. Furthermore, beads/photoresist scums also like to stick to bottom substrate after developing, which is very hard to remove even with ultrasonic agitation (Fig. 2 (b)). The solution is a bottom buffer photoresist layer spun before the mixture film, which prevents direct beads-to-substrate contact. In summary the bottom buffer photoresist, the beads/photoresist mixture, and the top buffer photoresist are spin-coated in sequence and patterned all-together.

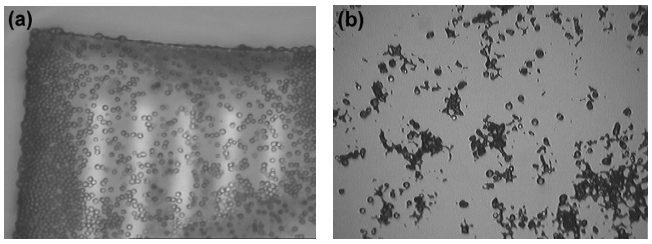

Figure 2. *Processing challenges. (a) Beads stick to top parylene if no top buffer photoresist is applied; (b) Beads/photoresist scums stick to substrate if no bottom buffer photoresist is used.*

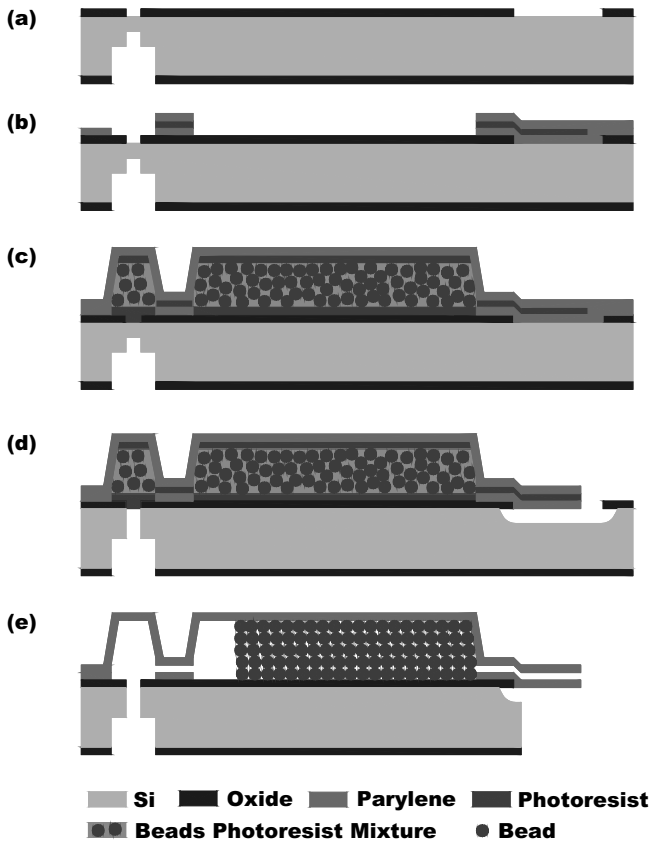

Figure 3. *Simplified process flow. (a) Pattern backside oxide and DRIE two-step backside holes; Pattern frontside oxide; (b) Deposit 1st parylene layer, pattern photoresist; Deposit 2nd parylene layer; Pattern the three layers; (c) Spin bottom buffer photoresist layer; Spin beads and photoresist mixture; Spin top buffer photoresist layer; Pattern the multiple photoresist layers; Deposit 3rd parylene layer. (d) Pattern parylene layers to open nozzle; DRIE opens backside holes; BrF_3 releases nozzle and makes trench (not shown). (e) Acetone dissolves photoresist; Break chip along trench to free nozzle; Pack the released beads into a column with pressurized flow.*

The thickness of the spin-coated mixture film depends on the spin rate, type of bead and photoresist used, and mixing ratio. The higher the beads content in the mixture, the thicker the resulting film, due to increase in the effective viscosity of the mixture. In order to successfully pattern the beads-embedded thick photoresist, multiple exposure-developing cycles may be needed. It is different from simply increasing exposure and developing time, in which only the mixture top part gets overexposed, since the beads may block the bottom parts from exposure. By developing away the exposed top part before the next exposure, the bottom parts of the photoresist can be fully exposed.

Despite the a few novel aspects detailed above, this process is still mainly a photolithography process, which allows its easy integration with other processes and/or devices. An integrated LC column –Electrospray Ionization (ESI) nozzle chip is fabricated by incorporating the above with a parylene ESI nozzle process similar to the one used in [**8**]. A simplified process flow is shown in Figure 3. The beads used are 5µm-diameter C18 porous silica beads, which is the most popular type for reverse-phase LC column. Photoresist AZ4620 was used to mix with beads at 1:10 (beads: photoresist) volume ratio. The mixture is spin-coated at 2.5krmp after a 2.5µm-thick bottom buffer photoresist coating and before a 5µm-thick top buffer photoresist coating. The total thickness of all photoresist layers is about 27µm. The filters height is 3µm. The fabricated column cross-section is 100µm wide by 26µm high, which shares the same cross-sectional area as a 58µm ID capillary. ESI nozzle freestanding overhang is 600µm in length. The nozzle opening is 3µm high by 15µm wide. The thicknesses of the parylene layers are 3µm (bottom), 3µm (middle), and 5µm (top). Figure 4 shows various parts of a fabricated LC-ESI chip, both before and after photoresist removal.

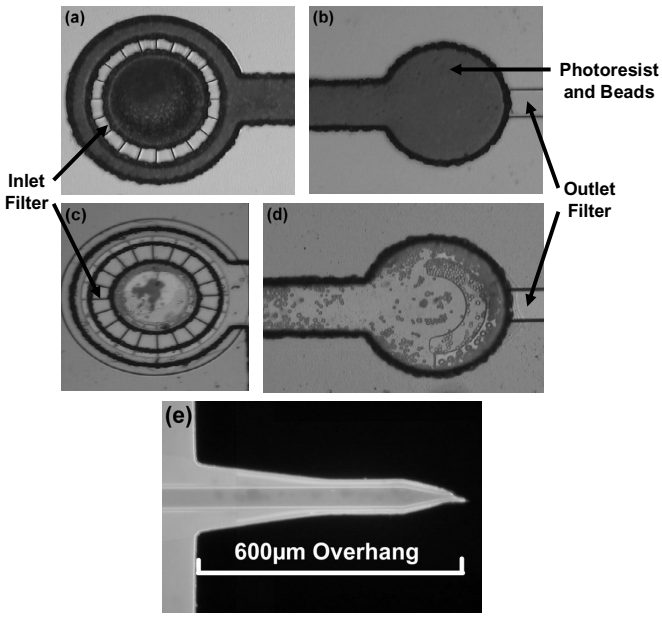

Figure 4. *Fabricated device pictures. (a) Column inlet and inlet filter before removing photoresist; (b) Column outlet to nozzle and outlet filter before release; (c) (d) After release, it can be seen that the beads are loosely released inside the channel; (e) Fluorescent picture of the freestanding parylene ESI nozzle. The nozzle opening*

is 3μm high by 15μm wide. The freestanding overhang part is 600μm long.

RESULTS AND DISCUSSION

While the beads are embedded in photoresist during processing, they are loosely released inside the column after photoresist removal. The beads can be easily packed into a dense column with a pressure flow (e.g. 15psi water flow). Figure 5 shows series of photomicrograph of released beads moving in a channel.

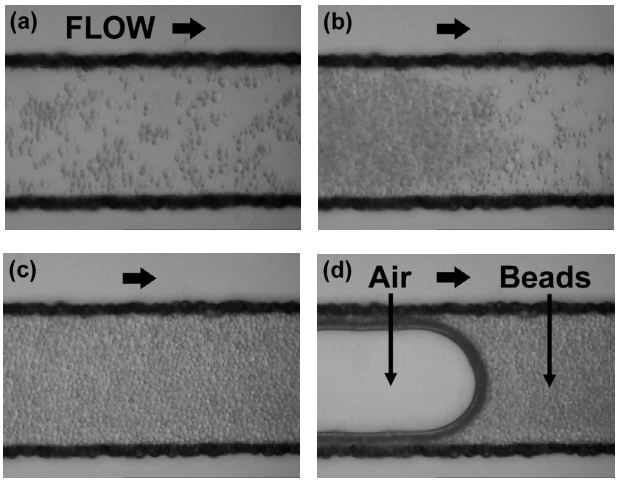

Figure 5. *Series snapshots of released beads moving with liquid from left to right in a microchannel. The channel is 100μm wide and 26μm high. Beads diameter is 5μm; (a), (b), (c) Beads move with liquid; (d) Air pushes liquid and beads.*

Figure 6 shows the column packing process and electrospray to a Mass Spectrometer. The full channel length (L_f) is 6800μm (Fig. 6 (a)). Since the nozzle end is a much smaller opening than the column inlet end, the beads pack to some degree at the nozzle end automatically during drying of the column, as shown in Figure 6 (b). After packing (Fig. 6 (c)), the LC column length (L_c) is 2700μm, which corresponds to 40% packing ratio (L_c/L_f). This ratio means 1cm-long channel with beads loosely released inside will turn into 4mm-long column heavily packed with beads. The ratio is dependent on original beads/photoresist mixing ratio. The higher the beads content in the mixture, the higher the packing ratio. The current 40% packing ratio is for original 1:10 beads-to-photoresist volume mixing ratio. To have long packed columns, large reservoirs instead of long serpentine channels can be used. The packing is done at 15psi with water. The flow rate during packing is on average 17nL/sec. After packing, the flow rate drops to 3nL/sec because of the increased flow resistance. The breakthrough time for an unretained sample to travel through this column and nozzle is 2.3sec. And because of integration, the post column volume is only 0.05nl including outlet filter and nozzle. Using the original mixing ratio and column packing ratio, the porosity of the bead column is estimated to be 0.75, which is consistent with externally-packed capillary columns (Fig. 8 (a)). The testing results of the column physical properties are summarized in Table 1.

The electrospray test is performed at 15psi with 98:2 Acetonitrile/water and 0.1% formic acid. The electrospray voltage of 1.2kV is applied off-chip at the upstream using the method described in [**9**]. When the voltage is off, the liquids coming out the nozzle form a droplet (Fig. 6(d)). When the ESI voltage is on, the sample liquid electro-sprays into the Mass Spectrometer. A typical electrospray figure obtained is shown in Figure 7. An eight-hour continuous spray testing is done without failure of the nozzle or observable nozzle performance degradation.

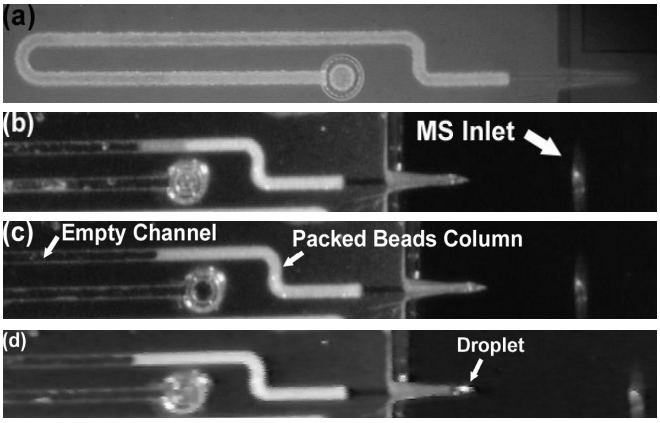

Figure 6. *Snapshots of packing a column and electrospray to a Mass Spectrometer (MS). (a) Fluorescent picture of the device used; (b) Before packing; (c) After packing; (d) Droplet forms at nozzle tip when electrospray voltage is off.*

	Column Properties
Beads Type	Porous Silica 5μm Diameter C18, pore size 100Å
Cross Section Dimension	100μm wide by 26μm high
Equivalent Column ID	58 μm
Full Channel Length (L_f)	6800 μm
Packed Column Length (L_c)	2700 μm
Packing Ratio (L_c/L_f)	40%
Flow Rate during Packing	17 nl/sec
Flow Rate after Packing	3 nl/sec
Breakthrough Time	2.3 sec
Post Column Volume	0.05 nl
Column Porosity	0.75

Table 1. *Summary of an on-chip column properties.*

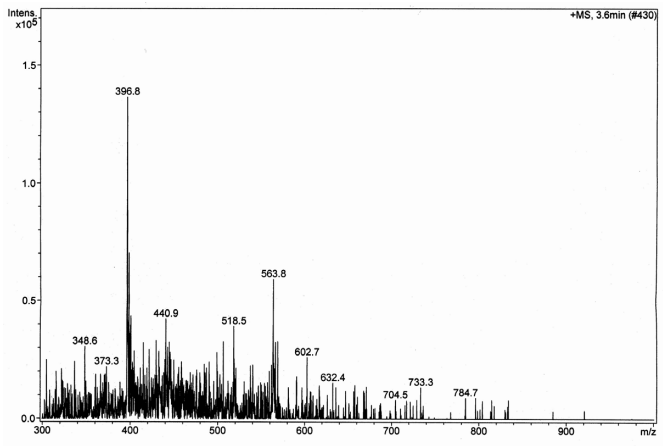

Figure 7. *A typical electrospray spectrum from the on-chip LC column and ESI nozzle.*

It is important to validate that the C18 silica beads function as normal after photoresist contamination and acetone cleaning. To do so, a capillary column-nozzle (Fig. 8(a)) packed with photoresist-

contaminated-then-acetone-cleaned C18 beads is used to separate Cytochrome C tryptic digest standard, using reverse-phase LC with gradient elution. The sample concentration is 0.1μM. Injection volume is 1μL. The MS data (Fig. 8 (b)) shows the column still achieves separation with performance comparable to similar capillary columns packed with fresh C18 beads. However, it is also found that the background noise level does depend on the cleanness of the column, as the background drops with more flushing cleaning of the beads column.

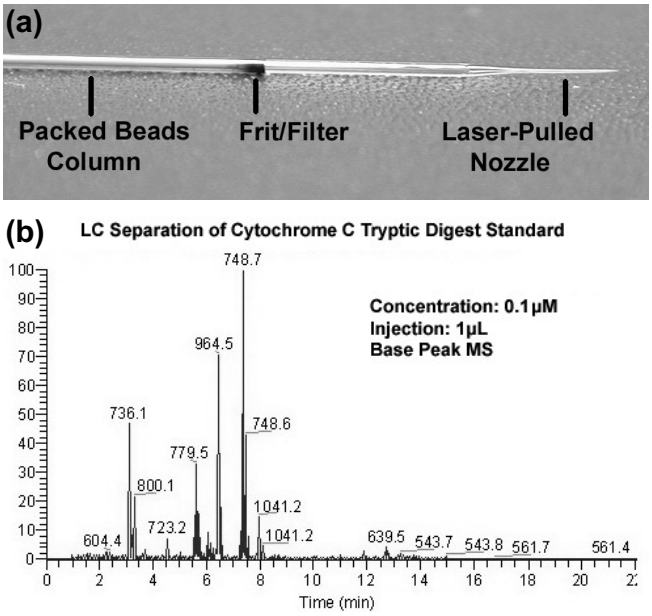

Figure 8. *(a) Picture of externally-packed capillary LC column and ESI nozzle; (b) MS data for LC separation of digested Cytochrome C using the capillary column packed with photoresist-contaminated-then-cleaned C18 beads.*

Future work includes performing on-chip LC separation and ESI to MS for detection, and comparing the separation capability of on-chip columns to conventional capillary columns.

CONCLUSIONS

In summary, a novel method to integrate beads into micromachined devices has been developed, which is wafer-scale batch processing, can be easily incorporated into other processes, and does not depend on bead types. This process is used to make an LC column – ESI nozzle on-a-chip. The column physical properties have been characterized. The ESI nozzle functions without problem in eight-hour continuous spray. Although the integrated beads have been contaminated with photoresist during device fabrication, their separation performance is not found to be affected, using a capillary column packed with contaminated-then-cleaned LC beads.

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