ION-PAIR REVERSED PHASE LIQUID CHROMATOGRAPHY OF DNA IN DEEP-UV PATTERNED SILICON PILLAR ARRAYS

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ABSTRACT

The ion-pair reversed phase (IP-RP) separation of short, similarly sized, double stranded (ds) DNA strains is demonstrated in a functionalized silicon pillar array channel with 1 µm flow-through pores. Using distribution zones and embedded sidewall regions optimized through computational fluid dynamics simulations, 3 nl DNA sample volumes could be transported into the pillar array with virtually no injection related dispersion. During the subsequent isocratic separation, a sequence of 5 ds DNA strains with length differences of 50 base pairs could be separated within 5 s, using only 1 mm of the channel length.

KEYWORDS: DNA chromatography, pillar array column, collocated monolithic support structure

INTRODUCTION

In the last few years, the pillar array column format has come into consideration as a serious and viable alternative for both the packed bed and the monolithic column in the field of liquid chromatography. Where the flow resistance in the pillar column is comparable to that of monoliths, the ordered nature of the pillars allows for theoretical plate heights that are a factor of two smaller than those of disordered particulate columns with equally sized particles [1]. As the performance of a separation column scales inversely with the ‘particle’ dimensions, an important focus in the development of this format is the production of smaller and smaller pillars.

A reduction in feature sizes has an important impact on several aspects of the separation column. The depth that can be achieved between etched features is, e.g., limited by the smallest gap dimension, a maximum aspect ratio of 50 is the very best value that can be expected. This reduction in channel depth will adversely impact detection, as the amount of material reaching the detector becomes smaller. A strategy can be conceived to increase the channel’s cross section by using wider channels. Using wide channels with relatively small depth makes the injection procedure complex: it requires a uniform distribution of sample plugs that, furthermore, must be well defined in a volume on the chip of known dimensions and positioned close to the separation channel to avoid dispersion due to interfaces that contain stagnant fluid zones.

Another critical fabrication aspect of small pillars is that it is difficult to produce porous pillars. It is well recognized that most separation problems require specific surface areas of porous structures, as the concomitant retention factors are 1-2 orders of magnitudes larger than those of non-porous structures. It is to be expected that the most common strategies to generate porous layers in silicon pillars such as HF anodization and sol-gel-based techniques are not compatible with high aspect ratio sub-micron features as they will not result in mechanically stable and uniform layers [2,3].

Still non porous pillar arrays have interesting aspects, the first is that they are particularly suited for ion-pair reversed phase separation of relatively small (less than 1000 bp) DNA molecules [4]. As these molecules have a very slow mass transfer in the mesopores of porous particles, very low efficiencies are obtained in porous particle columns. In packed beds, polystyrene-divinylbenzene particles have been used as stationary material for this purpose. The second aspect is related to their small volume and their ease of on chip integration. Given the small quantities of DNA that are typically available, and the need to amplify, inject and detect the DNA all on the same (low dead volume) substrate, it is extremely appealing to perform DNA separations on-chip. This should not only improve the separation efficiency, but also pave the way towards integrated portable diagnostic devices. In comparison with electrophoresis, another separation technique prone to miniaturization and integration, chromatography has the advantage that the elution power during the course of an experiment can vary, while electrophoresis conditions are fixed. This feature allows tailoring the experimental conditions to the specific sample, hence minimizing the separation time.

In the current paper, a non-porous silicon pillar channel design and its fabrication strategy is presented and a proof-of-principle of the separation of a few ds DNA strains is demonstrated. We have performed separation experiments detecting into the channel directly. As the detection window can be placed wherever a separation occurs, for these relatively similar DNA strains there is no need to use gradient elution. This approach furthermore allows for a true straightforward comparison between different column formats.

EXPERIMENTAL

The device consists of three parts, an injection zone, a distributor and a separation region. The injection zone (see Fig. 1a) has a volume of 3 nl and is connected to the distributor by a 200 µm wide channel. The distributor connects this narrow zone to the wider (1mm) separation region, the flow is distributed in the radial direction by elongated pillars (Fig. 1b). The separation channel contains 1 µm wide and 10 µm long pillars that have a flow-through pore of 1 µm.

The fabrication process is briefly described hereafter. The pillar structures were first defined in 500 nm thick positive resist spun on a 8 inch silicon wafer using a 2 cm large deep-UV (193 nm) stepper mask, that was printed on the wafer...
using a stitching and blading-off scheme. Next, the structures were etched in a 400 nm thick oxide layer underneath. Injections regions and distributors were defined on the same oxide layers by standard contact lithography. Mobile and sample supply channels and pillars were simultaneously etched in the Si by using the Bosch® etching process and the oxide pattern as hard mask. The channel was sealed by anodic bonding of the processed wafer to a Pyrex® substrate. Further, another litho step using 24 μm thick resist defined the 800 μm diameter access holes which were then transferred through the silicon by Bosch® etching.

For the functionalization of the pillars, a solution of 5 % dodecyldimethylchlorosilane in toluene (Sigma-Aldrich, Belgium) was pumped through the pillar array for 24 h, leaving a hydrophobic monolayer on the pillars. This monolayer can then interact with the triethylamine (TEA) in the mobile phase solution during the experiments, which in turn can interact with the negatively charged DNA phosphodiester group through the protonated amine group. The injections and separations of the SybrGreen stained DNA were performed by using pressurized vessels and automated valves. Observations occurred with a fluorescence microscope and a CCD camera, the plugs were followed during their motion through the channels by using an automated translation stage on which the chip was mounted.

**RESULTS AND DISCUSSION**

The separation channel defined in the oxide mask is depicted in Fig. 2a. A cross section of the pillar array etched in the silicon is depicted in Fig. 2b. The pillars have an aspect ratio of 20, this is a compromise value that allows to achieve a relatively deep channel maximizing the fluorescence signal on the one hand, and to have a optimal separation efficiency on the other hand. As the pillars are slightly tapered, the flow-through pore is 200 nm less at the bottom of the channel, hence the local velocity of the top and the bottom fraction of the channel will be different. This results in an additional source of band broadening. Being stagnant zones, the top and bottom layer also give rise to an inherent dispersive effect which again becomes more important at higher aspect ratio’s, regardless of any taper effects.

A 3 nl sample plug is defined in the injection box (Fig. 1a) and injected into the separation channel. This small plug volume ensures a minimal space occupancy in the separation channel. A distributor region (Fig. 1b) that contains pillars elongated in the radial direction will subsequently transport this plug to the much wider separation channel.

**Figure 1:** a) On-chip injection area. The injection volume is typically on the order of 3 nl. The injection is effectuated by switching a valve that connects pressurized vessels with the chip, this either allows an injection flow or a mobile phase flow. b) Distributor area that enables a low-dispersion transition between the narrow (200 μm wide) injection zone and the wider (1mm) separation channel. The design was optimized by computational fluid dynamics [5].

**Figure 2:** Separation area a) Top-view after RIE etching of the oxide hard mask. b) Cross-section of the DRIE (Bosch-type) etched pillar channel after etching and removal of the oxide hard mask. The height of the structures is 20 μm, the distance between the features is 1.2 μm at the top and 1 μm at the bottom.
The effectiveness of this optimized distributor [5] and of the accurate determination of the critical ‘magical’ distance of pillars from the side walls, is clear in Fig. 3a-b, showing that separated plugs moving in the channels are narrow and have a straight front.

The separation performances of the fabricated device are demonstrated in Fig. 3. Fig. 3a-b refer to the separation of a mixture of DNA ds fragments of 200 and 300 base pair pairs. Separation is obtained within only 0.5 mm of the channel length. Fig. 3c shows the separation of a 5 component mixture, the separation is obtained in only 5s. In contrast with similar experiments described in the literature using traditional packed beds [4], the mobile phase composition was kept constant during the course of this experiment. This isocratic mode operation is possible because the DNA strains are relatively similar in size, and also because the chip system does not suffer from connectivity losses, which are instead present in gradient mode, where the separating element is typically coupled to an external injector and detector (connectivity losses in gradient mode are partially compensated by peak compression effects inherent to that operation mode). Isocratic operation is particularly useful in the development of a portable diagnostic DNA separation device, where the implementation of a gradient can be cumbersome.

CONCLUSION

A silicon pillar array that is capable of separating 5 ds DNA strains within 5 s, based on IP-RC liquid chromatography is presented. By using an integrated sample distribution structure, a well defined volume can be very efficiently injected in the much wider separation channel. This integrated system allows for the injection of sample plugs that are tiny in width (typically about 100 µm). The combination of the small initial plug width with the high efficiency of the pillar column allows a very fast operation.

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REFERENCES


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