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Short Communication

Micro vials on a silicon wafer for sample introduction in capillary electrophoresis*

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ABSTRACT

A multi-sample holder produced by photolithographic and anisotropic etching techniques on a monocrystalline silicon wafer is described. Well defined volumes are created by utilizing the crystal orientations of the silicon material. By using step and repeated lithography, a large number of identical cavities can be obtained on a single chip. The surface is covered with a thin film of gold, which serves as a cathode during electrokinetic injections. Evaluation was carried out by injecting a mixture of pd(A) oligonucleotides (40–60) bases from a 118-nl sample well on to a 50 μm I.D. polyacrylamide-filled gel column. When compared with injections from classical vials, no additional zone broadening was observed.

INTRODUCTION

Capillary electrophoresis (CE) has developed into a rapid, high-resolution separation method, capable of analysing ultra-small amounts of sample [1–4]. To maintain a high efficiency, only a very small sample volume (of the order of a few nanolitres) is injected into the capillary. However, such volumes are out of proportion, when compared to the size of the commonly employed injection vials. The vials have to be filled with much larger volumes (in the μl–ml range), which means that only a tiny portion of the sample is utilized for the analysis.

Problems occur when only very limited volumes of sample are available. Small sample droplets tend to stick to the walls of the vial at random positions [5]. Moreover, an electrode has to be dipped into the sample, which makes accurate probing very difficult. In such situations, the sample is therefore usually diluted, but this leads to reduced detectability. Hence there is a need for micro injection vials and improved handling techniques of micro samples in capillary electrophoresis. There are a growing number of applications, *e.g.*, in peptide mapping of proteins [6].

A receptacle for electrokinetic injection from mi-

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crolitre volumes has previously been described by Yin *et al.* [7]. They utilized a titanium rod electrode with a concave identation as a miniaturized sample cup for electro-driven sample introduction. The sample volume can be kept small, as the high-voltage electrode itself serves as the sample container. In molecular biological work, such as DNA analysis or micro polymerase chain reaction (PCR), a further reduction in the vial size and multi-sample capacity are of considerable interest. For precise manipulation of micro samples, stringent demands are imposed not only on the size uniformity of the vials, but also on the precision of their inter-geometrical location.

Three-dimensional micro structures can be machined with extreme precision in a wafer of single-crystal silicon with a technique called micro-machining [8]. This method is well known in the semiconductor industry and has been used for the fabrication of analytical micro GC columns and ancillary equipment [9,10]. In this paper we describe a multi-sample holder that was produced by photolithographic and anisotropic etching techniques in a silicon wafer. An array of well defined micro cavities was created. The surface of the multi-vial wafer was coated with a thin film of gold that served as an *in situ* electrode for electrokinetic injections.

EXPERIMENTAL

Production of the silicon micro vials

A silicon wafer [thickness 380 μm, diameter 3 in. (1 in. = 2.54 cm), ⟨100⟩ single crystal] (Wacker-Chemitronic, Burghausen, Germany) was first oxidized at 1100°C in an H_2O–O_2 gas atmosphere containing trace amounts of HCl. A surface of SiO_2 (0.82 μm thickness) was thereby produced. A photolithographic mask with evenly spaced rectangular non-UV transparent images (1.5 × 0.5 mm) was manufactured with a step and repeat camera. The wafer was spin-coated with a UV-curing negative resist (20 drops, 5000 rpm, 30 s). Next, the lithographic mask had to be carefully aligned towards the flat of the silicon wafer. Openings in the oxide layer were created by UV lithography followed by etching with buffered HF. Subsequently, the unprotected silicon was etched anisotropically with EDP (ethylenediamine–pyrocatecol–H_2O) at 115°C for 6 h and then with KOH (ambient temperature) overnight. This resulted in wedge-shaped cavities (Figs. 1 and 2), with a volume of 118 nl. Finally, the surface was covered with a sputtered film of chromium and gold (the chromium film was needed to ensure adherence of the gold film to the silicon wafer).

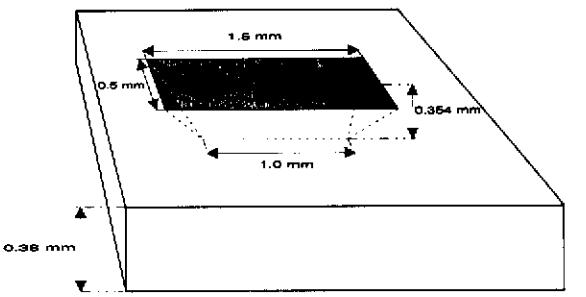

Fig. 1. Three-dimensional schematic diagram of a silicon micro vial. The vial walls are intersecting ⟨111⟩ silicon planes.

Sample introduction from the silicon micro vials

The cathode end of a high-voltage supply was clamped directly to the gold-plated chip. The micro vials were filled with *ca.* 0.1 μl of a sample consisting of single-stranded oligodeoxyadenylic acid [pd$(A)_{40-60}$; Pharmacia, Uppsala, Sweden] with a syringe. Electrokinetic injections where made by placing the end of a polyacrylamide gel-filled capillary [51 cm (38 cm to the detector) × 50 μm I.D. × 350 μm O.D.; 6% T, 3% C[a]] into the silicon vial and applying 5 kV for 1.5 s. The capillary inlet was then lifted back to a plastic vial (0.5 ml) filled with buffer for the electrophoretical run. The run voltage was 15 kV. The run buffer was 100 m*M* Tris–250 m*M* boric acid–7 *M* urea. On-column UV detection was performed at 260 nm. For evaluation of the performance, injections were also made from conventional plastic vials (0.75 ml volume) containing 50 μl of sample.

RESULTS AND DISCUSSION

In silicon micro-machining, thin-film techniques similar to those employed in the fabrication of integrated electronic circuits are used. A subsequent chemical etching adds a third dimension to the structures. Extremely well defined V-grooves can be

[a] C = g N,N'-Methylenebisacrylamide (Bis)/%T; T = (g acrylamide + g Bis)/100 ml solution.

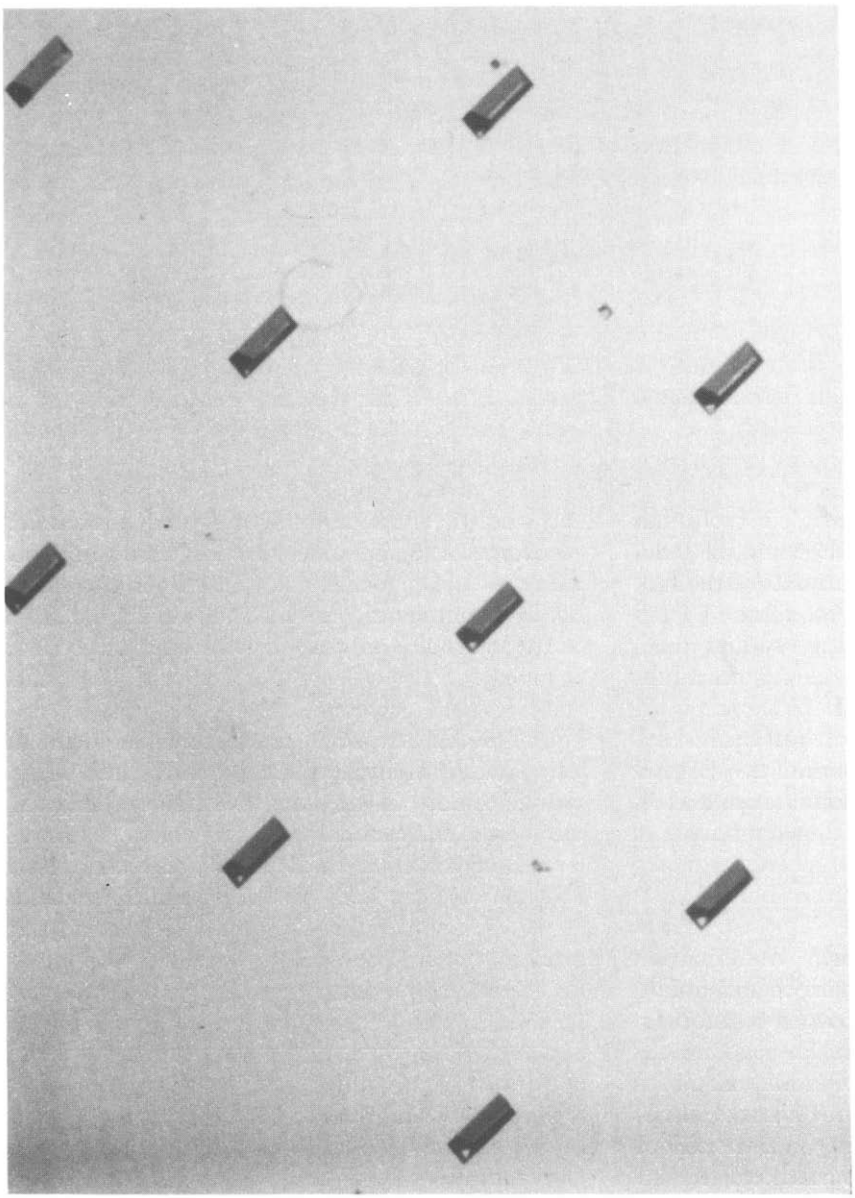

Fig. 2. Photomicrograph of the gold-coated micro vials. The distance between each vial is 7 mm.

created by careful alignment of the lithographic mask to a ⟨100⟩ oriented silicon wafer and using an anisotropic etchant, such as EDP or KOH. Relatively fast etching occurs in all crystal directions except in the ⟨111⟩ direction. Thus, the remaining intersecting ⟨111⟩ planes define our array of 354-μm deep holes with an inherent precision.

We are aware that silicon micro-machining techniques are not particularly familiar to chromatographers. We recommend refs. 8 and 9, where excellent overviews are given of some of the major features, techniques and possibilities of silicon-machining.

Fig. 3a and b shows the electropherograms ob-

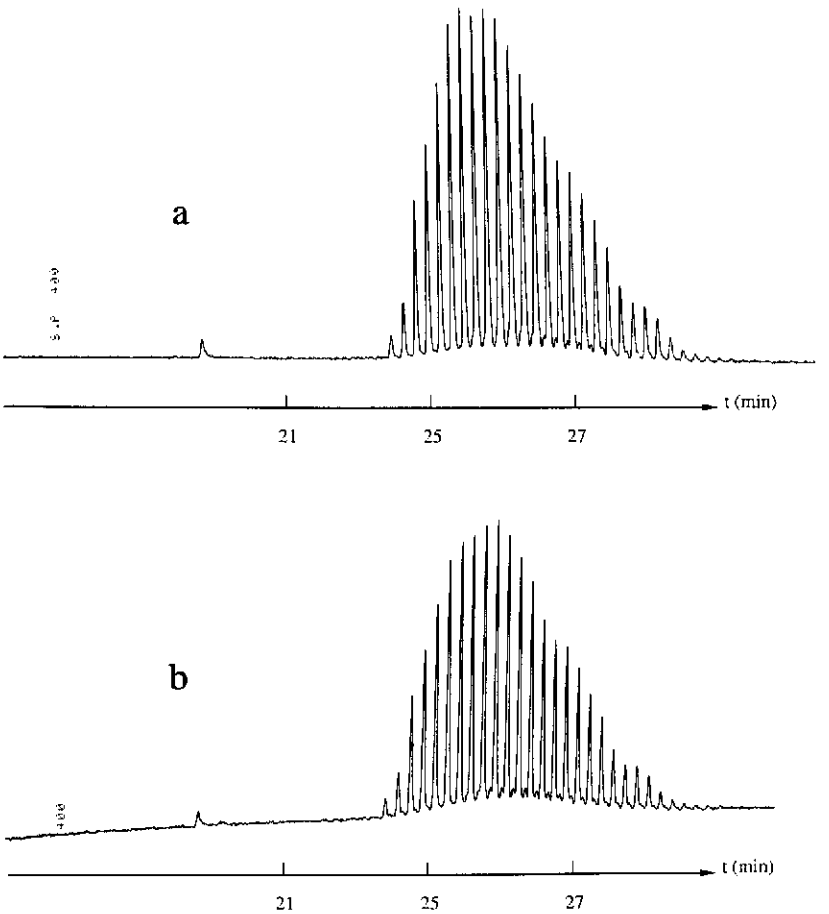

Fig. 3. (a) Separation of oligodeoxyadenylic acids pd(A)$_{40-60}$, electro-injected from a conventional plastic vial. For conditions, see text. (b) Separation as in (a) except that the electro-injection was made from a silicon micro vial.

tained after injection from a silicon micro vial and from a classical vial, respectively. As can be seen, the zone broadening is similar in both instances. The resolution between the twelfth and thirteenth peaks in the electropherogram was calculated according to

$$R = \frac{t_2 - t_1}{\sqrt{\frac{2}{\ln 2}} \cdot \frac{w_{h2} + w_{h1}}{2}} \quad (1)$$

where t_1 and t_2 are the retention times and w_{h1} and w_{h2} are the peak widths at half-height. The resolution was 2.39 for the vial-based injection and 2.58 for the injection from the silicon micro vial. Hence there is a slight improvement, but it was not possible to verify this statistically.

A drawback with electrokinetic injection from small sample volumes is that contamination by electrolysis products is more pronounced. A prolonged injection time should therefore be avoided. Another observation was that evaporation of water from the sample must be avoided, *e.g.*, by capping the holes with a thin membrane or by water-saturating the headspace [11].

The technique presented allows the fabrication of micro vials with nanometre precision. A large number of vials (*e.g.*, 1000 or more) can be etched on a single chip. With appropriate photolithographic

masks, picolitre vials can be made with high precision. The geometric location of the vials on the wafer can be defined at exact intervals, which facilitates automated sampling with micro-robotics [12]. The V-shaped bottom geometry also helps in positioning a microprobe in the vial. As silicon wafers can be batch processed in large amounts, identical micro-machined structures can be produced at low cost.

The concept of the surface-coated electrode has several advantages: the electrode is in a static position and common for all sample vials, which simplifies the design of the electrophoretic equipment and makes it possible to reduce further the vial volumes; cross-contamination is reduced, as no electrode is dipped into the sample; and the electric field is initiated at the vial surface and not from a conventional wire electrode so that sample molecules should be uniformly repelled from the surface, which is likely to be beneficial in reducing sample adsorption on the vial walls.

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