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High-Density Plastic Microfluidic Platforms for Capillary Electrophoresis Separation and High-Throughput Screening

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Modern drug discovery and genome analysis depend on the rapid analysis of a large numbers of samples in parallel. Microfluidic devices must be of low cost, to be applied in this field, which means that they have to be mass producted. A novel microfluidic platform of the standardized microplate footprint for applications in high-throughput drug screening (HTS), DNA and protein analysis has now been developed jointly by Greiner Bio-One and the Karlsruhe Research Center. Mechanical micromachining is used to manufacture a molding tool for replication in plastics, *e.g.*, polymethylmethacrylate (PMMA), polycarbonate (PC), cycloolefin copolymer (COC), polyoxymethylene (POM), polyetheretherketone (PEEK), polyvinylidenfluoride (PVDF), polysulfone (PSU), and polystyrene (PS). In low-cost mass production, plastic microfluidic devices with a high density of microchannels are fabricated by vacuum hot embossing. The microchannel arrays are sealed simultaneously with a one-piece cover plate by applying a unique bonding technology to ensure reliable applications without any leakage or cross flow.

1. Introduction

In the course of drug development in the pharmaceutical industry, more than 100,000 substances are tested for effectiveness every week. During the mean drug development

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time of approximately 10 years, large amounts of reactants are consumed, while the costs incurred amount to some 100 million Euro.[1]

For a considerable reduction in costs and development time, the reagent volume is constantly decreased. The measurements are performed with a maximum of processes taking place in parallel. However, in case of sample volumes of less than 1 μl, evaporation losses become evident such that work can no longer be carried out with open test vessels. Closed microchannels represent the solution to this evaporation problem. They also allow for a variety of processing options, such as mixing and separation of tiny sample volumes. Separation of *e.g.*, DNA and protein mixtures in microchannels is achieved far more rapidly than in glass capillaries or agarose gels.[2]

Microfluidic devices fabricated by mass production offer numerous application potentials, such as high-throughput drug screening, clinical diagnostics, and gene analysis.[3] The low unit production costs of plastic substrates make it possible to produce single-use devices, thus eliminating the need for cleaning and reuse. Combination of low assay volumes and integrated capillary electrophoretic separation represents a powerful tool for rapid assay development.

Microfluidic devices can be produced by microtechnical fabrication processes in combination with plastic molding techniques.[6] In principle, replication in plastics requires a hot embossing or injection molding tool. Various microfabrication technologies for master fabrication have already been established, such as mechanical micromachining, micro electrical discharge machining (μEDM), and the LIGA technique. Depending on the specific requirements, the best suited process is selected. All technologies yield robust metal molding tools which exhibit the inverse shapes of the intended microstructures.

So far, microchannel structures have mostly been produced on small areas (less than 4 inches in diameter), and mainly in glass or silicon using conventional etching techniques. However, transparent plastics are far less expensive than glass or silicon, numerous materials are available for selection, and molding techniques exist for the low-cost mass production of microfluidic components on large areas.

2. Master Fabrication

Master fabrication assumes a key position in plastic replication technology. Depending on the product requirements, i.e., structural dimensions, accuracy or fabrication costs, different master fabrication technologies are applied.[4]

Micromilling techniques[5] enable the production of structures of down to 50 μm in dimension. Microerosion techniques[6] may be applied for the fabrication of even smaller structures. Laser ablation[7] is another machining method with minimum dimensions of about 5 μm being reached.

Silicon can be microstructured by means of etching techniques. Due to its brittleness, it is not suited for direct use as a mold insert material. Subsequent electrodeposition of metal is required to produce microstructures as small as 2 μm in size.[8] By means of the LIGA technique,[9] a lithographic microfabrication method developed at the Karlsruhe Research Center, mold inserts with smallest structures are produced. A combination of this technique with ultraviolet lithography or X-ray lithography yields structures of down to 5

μm or 0.2 μm in size, respectively. Of all mold insert fabrication techniques mentioned, micromilling and microerosion are associated with the smallest expenditure in terms of time and cost. Complex mold inserts are frequently produced by a combination of various microfabrication methods.

3. Plastic Replication

Microfluidic devices made of plastic materials offer many advantages in comparison to microstructured glass or silicon devices. There is a huge variety of resins available and polymer materials from the shelf may be obtained at reasonable prices. Additionally, the surfaces of plastic materials are biocompatible or can be treated or coated to achieve biocompatibility.

Using a single metal mold insert, thousands of microstructured plastic products can be manufactured by molding techniques, *e.g.*, hot embossing and microinjection molding. Both techniques are suited for mass production. A variety of plastics, i.e., PMMA, PC, COC, POM, PEEK, PVDF, PSU, and PS, can be employed.

In hot embossing,[10] the microstructured mold insert is evacuated and pressed into a plastic plate, the mold having been heated slightly above the glass transition temperature of the plastic material. Thus, the microdepressions of the mold insert are filled by the plastic material. Then, the mold insert is cooled below the glass transition temperature until the plastic material starts to solidify again. Upon the removal of the mold insert, the embossed plastic plate is obtained. Due to the short flow paths of the plastic material, the hot embossing process enables precise replication of even smallest contours less than 1 μm in size.

In contrast to this, microinjection molding[11] is based on the melting of plastic granules in an injection unit. The resulting molding mass is injected into an evacuated and heated injection molding tool. This tool is provided with an integrated microstructured mold insert that is filled completely by the molding mass. Following cooling and opening of the tool, the injection-molded plastic components are ejected. Compared to hot embossing, injection molding usually allows shorter cycle times to be reached. However, investment costs of the tool and machine are higher.

A process that is mainly used in the academic world is the casting of silicone-based elastomers,[12] i.e., polydimethylsiloxane (PDMS), by pouring the elastomer and a curing agent over a microstructured template. After curing, the soft elastomer copy can simply be peeled off the mold and placed against a planar surface, to form closed channels. This technique offers flexible and low-cost access to planar microchannel structures.

4. Bonding Technology

Functioning of the microchannel structures depends decisively on their reliable and leak-tight sealing. Impurities or adhesives must not enter the channels. Deformation of the channel geometry has to be excluded. Open bonding gaps, the preferable locations of *e.g.*, adherent biomolecules, must be avoided. Various technologies, such as laser welding, ultrasonic bonding, adhesive bonding techniques, and diffusion bonding, are available.

5. Microfluidic Plate with 96 CE-Structures

The first example shows a microplate design which accommodates 96 identical microfluidic structures for separation by capillary electrophoresis (CE) in a standardized footprint[13] (Figs. 1, 2).

The inverse channel structures of the 96-channel CE-plates are milled into a large-area (128 mm × 85 mm) mold insert made of brass. Elevations in the mold insert correspond to the later channels in the molded plastic plate (Fig. 3). The areas between the protruding inverse channel structures are machined using finger mills of 50 μm to 400 μm in diameter. It is a particular challenge to minimize the round curves generated by the mills at the later

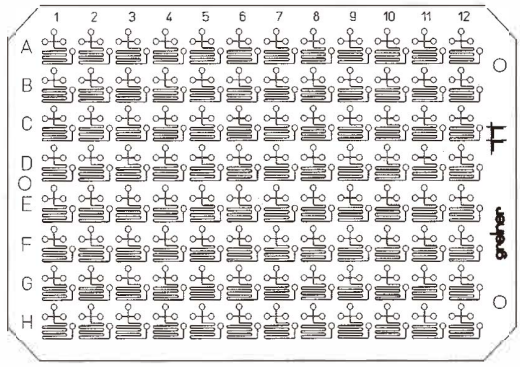

Fig. 1. Design of a standard microplate with 96 CE-structures.

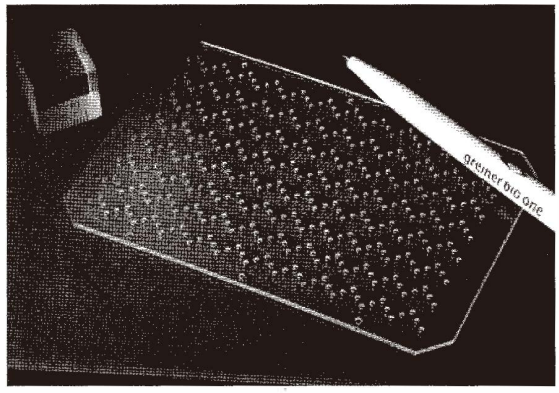

Fig. 2. Microplate with 96 CE microchannel structures.

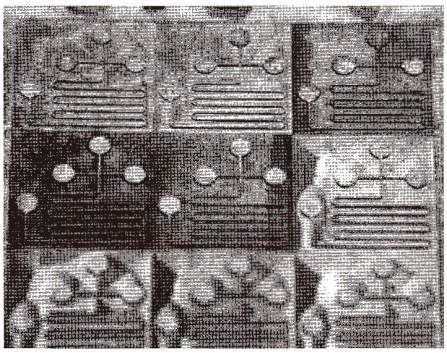

Fig. 3. Inverse micromachined CE-structures in brass.

channel crossings. So far, smallest radii of 25 μm have been reached. Further developed milling techniques already allow radii of 5 to 10 μm to be achieved at certain structural edges.

Each CE-structure consists of two crossing microchannels with reservoir openings at their ends. The cross sections of all microchannels are 100 μm $\times$ 50 μm. The reservoirs have a diameter of 1.4 mm and a volume of 1 μl. To conserve space, each separation channel is curved. Each separation channel has a length of about 40 mm (Fig. 4). The intersection volume in the intersection area of both microchannels amounts to about 600 pl (Fig. 5). The reservoir spacing is microplate-compatible with pitches of 9 mm, 4.5 mm, and 2.25 mm, respectively. Subsequent assembly in a standardized plate holder makes these devices well suited for automated screening and liquid handling robotics.

The cross section of a sealed CE-microstructure (Figs. 6, 7) reveals the successful bonding of the microchannels without any gaps and dead spaces being found. Tightness of all microchannels is confirmed by means of fluidic tests. In Fig. 8 a leakage test of a sealed CE-structure is demonstrated. A droplet of a red-colored aqueous dye is dispensed in one of the reservoirs. The liquid moves immediately into the channels by capillary forces. After approximately 12 s, the microchannels are filled completely with the dye. Leaving the dye for several minutes in the channels shows that no creeping of liquid occurs at the interface. Neither leakage nor short-cuts between adjacent loops can be seen. Successful fluid testing of several CE-structures demonstrates the perfect sealing of the microchannels.

Electrokinetic microchannel injection of fluorescent DNA is demonstrated by applying electric potentials to the reservoirs (Fig. 9).

6. Electric Conduction Paths

To perform CE in up to 96 microchannels in parallel, conducting paths are integrated in the microfluidic plate.

Microeroded metal masks are used for physical vapor deposition (PVD) of gold

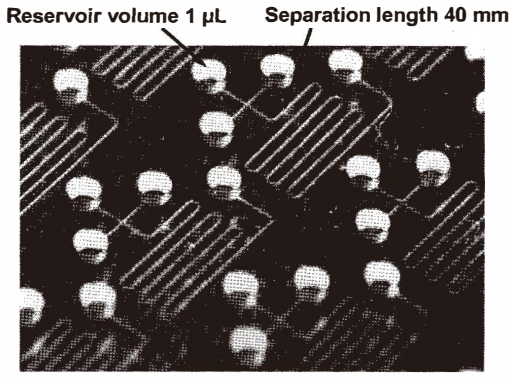

Fig. 4. Molded and sealed CE-structures in PMMA.

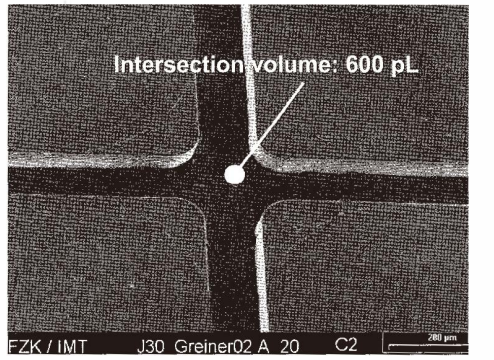

Fig. 5. Intersection of two microchannels molded in PMMA.

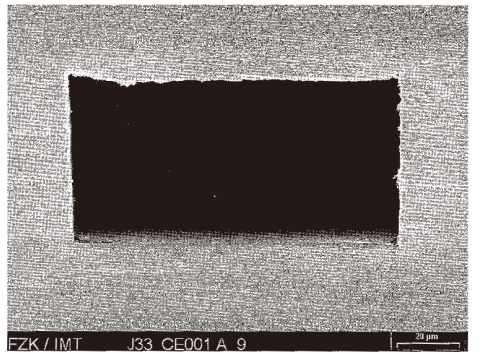

Fig. 6. Cross section of a sealed microchannel (50 μm×100 μm).

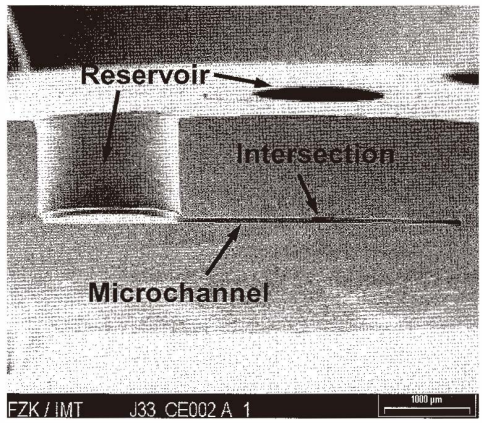

Fig. 7. Cross section of a reservoir, the outgoing microchannel, the intersection area, and the entry area of the meandering separation channel.

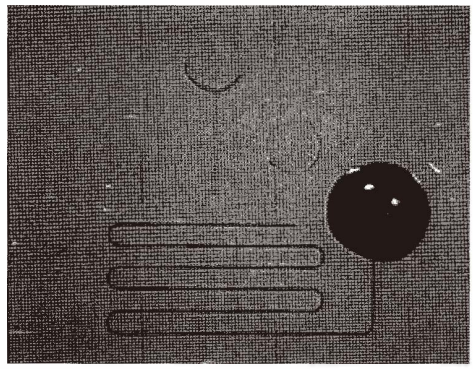

Fig. 8. Leakage test of a sealed CE-structure.

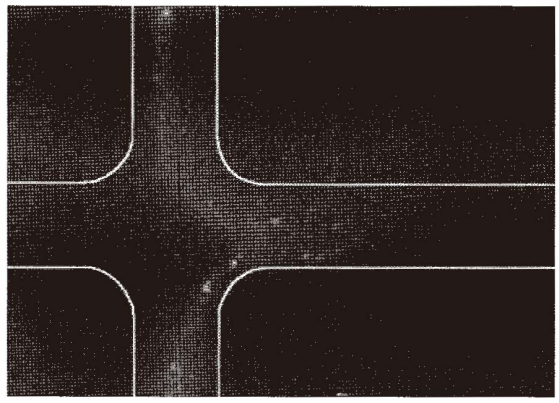

Fig. 9. Electrokinetic microchannel injection of fluorescent DNA.

microelectrodes to connect the reservoirs electrically from the rim of the 96-channel microfluidic plate (Fig. 10). To avoid electric contact at the crossings of the conduction paths, the 200-μm-wide and 400-nm-thick conductors run in two different layers. The upper layer is provided with a conductive coating at the bottom and the side walls of each reservoir. The microelectrode design enables electric contacting of a single CE-structure or up to 96 CE-structures in parallel.

Electric contacting of the 384 openings (reservoirs) takes place via conduction paths that are integrated in the microstructured plastic plate (Fig. 10). Sample volumes of less than 1 μl can be pipetted automatically into the reservoirs and a substance volume of less than 1 nl may be separated in each CE-structure. 96 CE-separations may take place in parallel.

For the defined motion and separation of the substance mixture to be analyzed, electric voltages are applied to the microtiter plate from outside. Via the conduction paths, these voltages are passed on to the reservoirs. Upon the separation of the substance mixture, the individual constituents can be detected optically at the end of the separation channel.

7. Microfluidic Plate with 384 Microchannels

The second example (Figs. 11, 12) illustrates a plastic microplate which contains 384 identical microfluidic structures with 384 integrated membranes. The microchannels are 250 μm wide and 350 μm deep, the reservoirs are 1.0 mm and 1.4 mm in diameter, and the membrane pieces are 1.0×1.8 mm^2 in size. The reservoirs have a 2.25 mm pitch. By applying an electrical potential between the end reservoirs at each channel, peptides are separated by charge, and the negatively charged peptides get captured at the membrane. The signal is detected using a commercial fluorescence plate reader.

Assembled in a standard microplate frame, the plates can be processed and read by commercially available instrumentation. The time and reagent volume needed to run assays are decreased significantly.

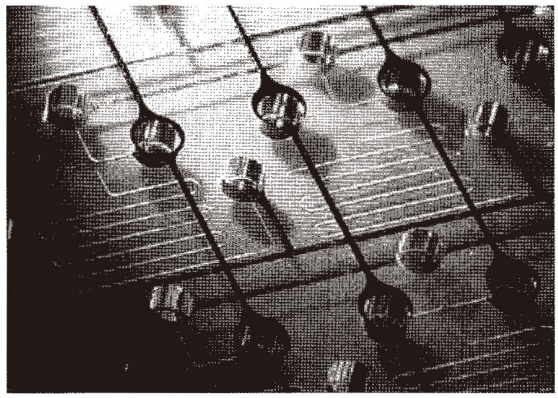

Fig. 10. Electrical conduction paths in two different layers.

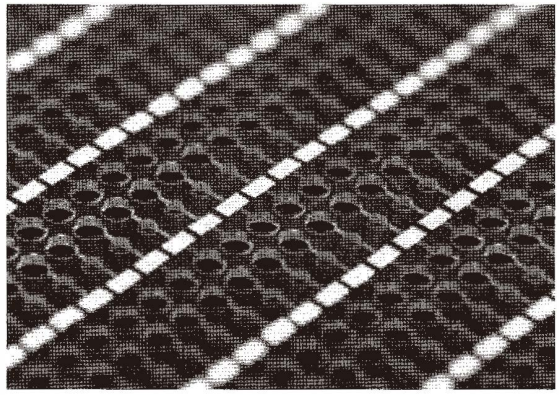

Fig. 11. Microplate with 384 microfluidic structures.

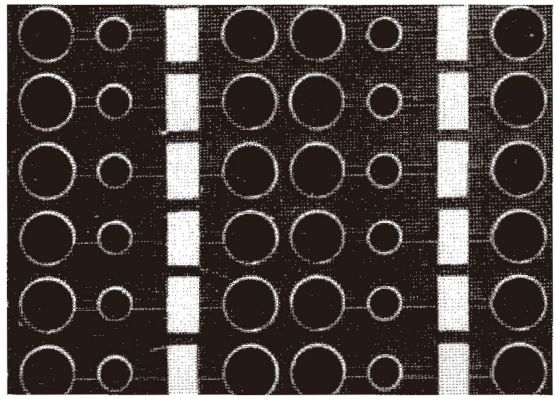

Fig. 12. Detail of 384 microchannels with integrated membranes.

8. Conclusions

For the first time, microplates with integrated microchannels, conduction paths and membranes have been produced by large-area (128 mm × 85 mm) plastic molding.

Master fabrication techniques are well established to generate microstructural dimensions of less than 50 μm. Hot embossing allows low-cost mass production of single-use plastic microfluidic devices. The feasibility of structuring large areas with 96 and 384 CE-microchannels on a standardized microplate made by hot embossing has been demonstrated. Our sealing technology for leakage-free covering of microchannels – even with

integrated membrane pieces – has been proved successfully. The microfluidic plates can be applied for high-throughput screening, DNA and protein analysis.

Handling and analysis of these novel microtiter plates are further simplified by integrating microelectrode structures as demonstrated by the 96 CE-structure plate.

Microfluidic channel structures have the potential of integrating various processes, i.e., sample collection, pretreatment, amplification, hybridization, and detection, in a single platform. Significant advantages are the increased speed, reduced costs, smaller reagent consumption, increased efficiency and automation. Enormous cost reductions in high-throughput screening result from the parallel arrangement of microchannel structures on the standard microplate format.

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