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Microfabrication of single-use plastic microfluidic devices for high-throughput screening and DNA analysis

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Abstract Modern drug discovery and genomic analysis depend on rapid analysis of large numbers of samples in parallel. The applicability of microfluidic devices in this field needs low cost devices, which can be fabricated in mass production. In close collaboration, Greiner Bio-One and Forschungszentrum Karlsruhe have developed a single-use plastic microfluidic capillary electrophoresis (CE) array in the standardized microplate footprint. Feasibility studies have shown that hot embossing with a mechanical micromachined molding tool is the appropriate technology for low cost mass fabrication. A subsequent sealing of the microchannels allows sub-microliter sample volumes in 96-channel multiplexed microstructures.

1 Introduction

Microfluidic devices fabricated by mass production offer an immense potential of applications such as high-throughput drug screening, clinical diagnostics and gene analysis [1]. The low unit production costs of plastic substrates make it possible to produce single-use devices, eliminating the need for cleaning and reuse [2]. The combination of small assay volumes and the possibilities of integrated capillary electrophoretic separation provide a powerful tool for rapid assay development.

Fabrication of microfluidic devices can be applied by microtechnical fabrication processes in combination with plastic molding techniques [3]. Basically, replication in plastics requires a hot embossing or injection molding tool. Various microfabrication technologies for the master fabrication are established, such as mechanical micromachining [6], micro electrical discharge machining (μEDM) [3] and the LIGA technique [4]. Depending on the specific requirements, the most suitable process can be selected. The availability of these technologies allows to generate robust metal molding tools which exhibit the inverse shapes of the intended microstructures.

This paper will show a low cost production of 96 channel plastic microfluidic devices including various microfabrication technologies to demonstrate the application of microtechnical fabrication processes for high-throughput screening and DNA analysis.

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2 Master fabrication

The master fabrication holds a key position in plastic replication technology. Depending on the product requirements like structure dimensions, accuracy or fabrication costs, different master fabrication technologies are applied [5]. With mechanical micromachining [6] structural dimensions down to 50 μm are obtained. Micro electrical discharge machining [3] is a corrosive machining technique supported by an electrical potential. The smallest structural dimensions achieved with this technique are approximately 10 μm. Laser ablation can be applied to microstructure many materials. Structural dimensions down to 5 μm are achievable [7]. For the master fabrication with silicon micromachining [8] a subsequent electroplating step is required, because of the brittleness of the silicon material. Microstructures down to 2 μm can be produced with this technique. The master fabrication process with the highest structural resolution is the LIGA-technique [4]. Structural dimensions of approximately 5 μm are achieved with UV-lithography and down to 0.2 μm with X-ray lithography.

Mechanical micromachining is the least time and cost consuming master fabrication technology. In several cases complex masters are built up by combining the different microfabrication processes.

3 Plastic replication

Microfluidic devices out of plastic materials offer many advantages in comparison to microstructured glass or silicon devices. There is a huge diversity of resins available and the polymeric materials out of the shelf have reasonable prices. Additionally, the surfaces of plastic materials are biocompatible or can be treated or coated to achieve biocompatiblity.

Plastic materials can be microstructured by a single master fabrication and subsequent replication by injection

molding or hot embossing. From one single master thousands of plastic replicates can be manufactured.

Hot embossing is a superior tool for mass production of plastic microcomponents (Fig. 1). In this process, a microstructured mold insert is pressed into a thermoplastic polymer film under vacuum, the film having been heated beyond its glass transition temperature. The polymer fills the mold insert, creating a detailed inverted replica of the microstructures. Subsequently, the entire molding setup is cooled down, and the replicated structure is demolded from the mold insert [9].

As a consequence of negligible flow lengths and the use of low molding speeds, this embossing process allows even filigree microstructures with very high aspect ratios to be produced. Most thermoplastic materials are eligible for this process like PMMA, PC, POM, PEEK, PVDF, and PSU. At the present, only this technology allows for a very precise generation of the filigree channel structures on large-area plastic substrates.

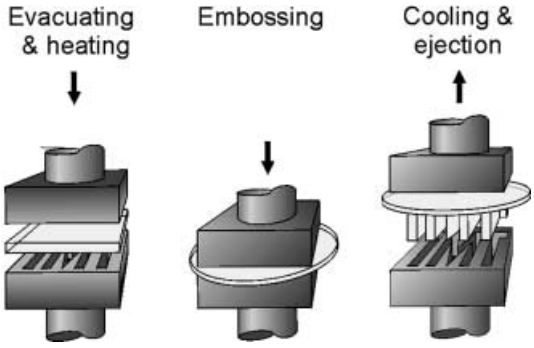

Fig. 1. Hot embossing for replication of various plastics, like PMMA, PC, POM, PEEK, PVDF, PSU

4 Fabrication and testing of a 96-channel plastic CE-plate

We have fabricated prototype single-use plastic microfluidic devices in a standard microplate format (Figs. 2, 3) by hot embossing with a mechanical micromachined molding tool and subsequent sealing of the microchannels (Figs. 7–10).

The molding tool is fabricated out of a large area brass plate by using various finger mills between 50 and 400 μm (Fig. 4). The microfluidic channels are embossed in plastic

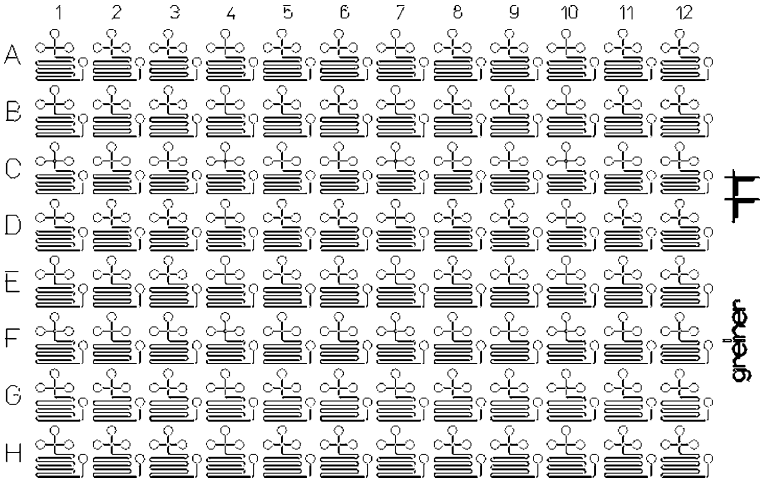

Fig. 2. Design of a standard microplate with 96 CE-structures

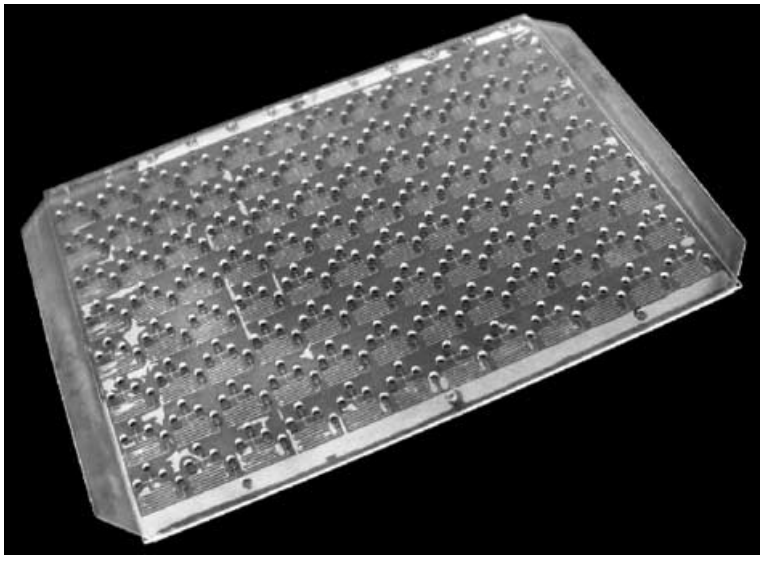

Fig. 3. Sealed microplate with 96 CE-structures

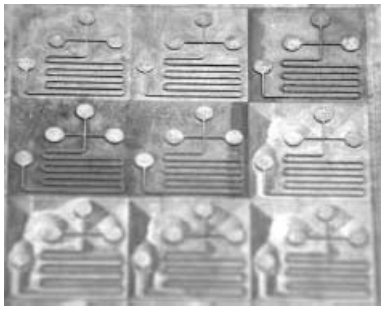

Fig. 4. Inverse micromachined CE-structures in brass

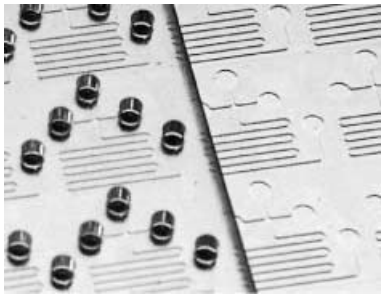

Fig. 5. Molded and partly sealed CE-structures in PMMA

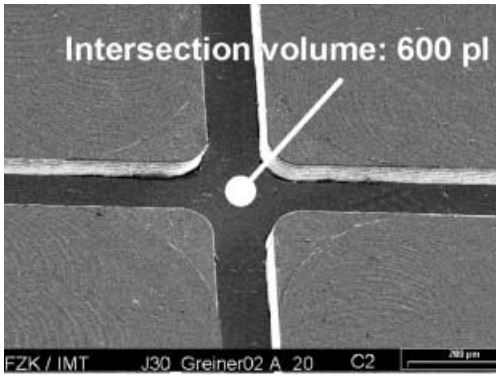

Fig. 6. Crossing of two microchannels

materials like PMMA, PC or COC (Figs. 5, 6). A complete embossing cycle takes about 15 min. The microchannels are sealed successfully with a plastic cover plate containing drilled holes on top of the reservoir positions (Figs. 3, 9).

This microfluidic plate enables capillary electrophoresis (CE) to be performed in 96 channels in parallel. The size of the CE-microplate matches with the standardized microplate format (approximately 128 × 85 mm). The microfluidic structures are compatible with existing plate and liquid handling robotics. The 96 CE-sections have a 9 mm lateral pitch and the reservoirs are positioned at a 2.25 and 4.5 mm lateral distance, according to the 1536 and 384-microplate format. In test measurements a plug of fluorescence marked DNA was electrokinetically injected into the gel filled separation channel and was clearly detected with a fluorescence microscope.

Each CE-structure consists of two crossing microchannel structures with a channel cross section of 100 × 50 μm. In the four buffer and sample reservoirs sample volumes

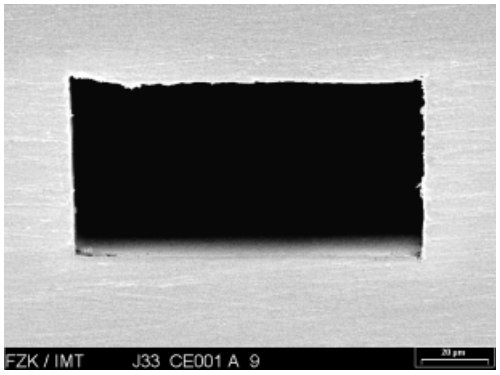

Fig. 7. Cross section of a sealed micro-channel (50 × 100 μm)

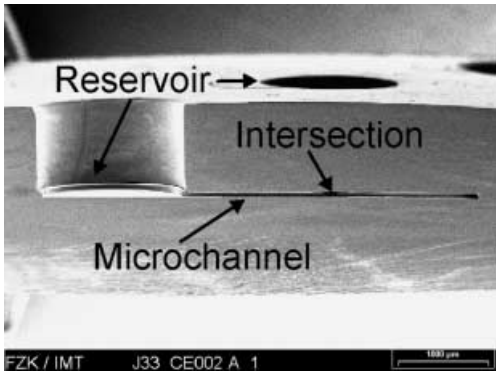

Fig. 8. Cross section of a reservoir, the outgoing microchannel, the intersection area, and the entry area of the meandering separation channel

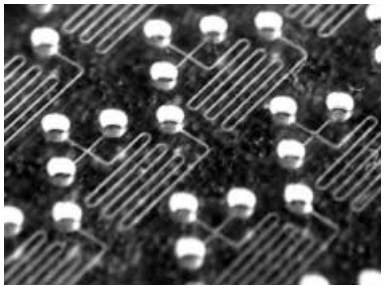

Fig. 9. Molded and sealed CE-structures in PMMA

between 0.1 and 1 μl can be applied, depending on the thickness of the cover plate. At the intersection of the two microchannels, a defined analyte volume of 600 pl is generated (Fig. 6). The downstream separation channel is arranged in a meandering manner and has a total length of 40 mm to ensure a fast and efficient separation (Fig. 5).

If necessary, the design of the microfluidic CE structures still can be varied over a wide range. In the curve areas, for instance, tapered channel geometries appear feasible. Meanwhile, mechanical micromachining allows to cut even finer web or groove structures. During subsequent manufacturing steps, they can be modified to mold inserts.

In Fig. 10 a leakage test of a sealed CE-structure is demonstrated. A droplet of a red colored aqueous dye is

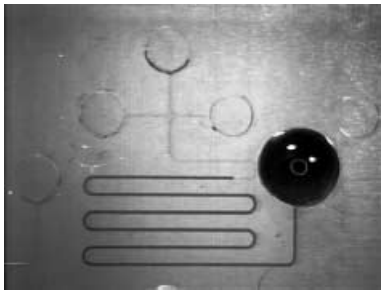

Fig. 10. Leakage test of a sealed CE-structure

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. The photograph shows that there is no creeping of liquid in the interface. Neither leakage nor any short-cuts between adjacent loops can be seen. The successful fluid testing of several CE-structures demonstrates the perfect sealing of the microchannels.

5 Conclusions

Master fabrication techniques are well established to generate microstructural dimensions of less than 50 μm. Hot embossing provides a low cost mass production of single-use plastic microfluidic devices. The feasibility to structure large areas with 96 CE-microchannels in a standardized microplate made by hot embossing has been demonstrated. Our sealing technology to cover microchannels without leakage could be successfully proofed. The 96 CE microfluidic plate can be applied for high-throughput screening and DNA analysis.

Handling and analysis of these novel microtiter plates may be further simplified by integrating the total of 384 microelectrode structures in all 96 CE-structures. For an increase in throughput and a reduction of the cycle time injection molding is envisaged.

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