A HIGHLY PARALLEL NANOLITER DISPENSING SYSTEM FABRICATED BY HIGH-SPEED MICROMILLING OF POLYMERS

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Abstract:
We present a highly parallel nanoliter dispenser for applications in high throughput screening (HTS) fabricated by high-speed micromilling in different plastic materials (PMMA, COC). The device according to the DWP principle [1, 2] consists of 384 microstructured dispensing units arrayed on a macroscopic area in the format of a standard micro well plate (approx. 80 x 120 mm). Each dispensing unit consists of 3 basic elements: a reservoir, a connection channel and a tapered nozzle. The challenge addressed in this paper has been the fabrication of those elements in a real 3D-geometry on a 1 mm thick polymer substrate by high-speed micromilling. We successfully fabricated microstructures with a width of 50 µm to 300 µm and an aspect ratio of up to 2.5. Measurements proved tolerances less than 5 µm leading to a droplet homogeneity of 10 % at a mean dosage volume of 60 nL.

Keywords: nanoliter, dispenser, micromilling, high througput screening

Introduction

Pharmaceutical research experiences an increasing need for highly parallel dispensing technologies in the nanoliter range to speed up drug development. For testing new compounds in well plates about 1,000 dispensing cycles per minute can be handled today. In order to improve this throughput by a factor 10 to 100 we developed the Dispensing Well Plate (DWP) [1, 2]. Suitable to the format of standard micro well plates it is able to deliver up to 1,536 different liquids in the volume range of 10 nL to 1000 nL simultaneously by a pneumatic actuation.
Micro well plates are usually disposables made by injection moulding of polymers in high quantities. Also the DWP will be used in its final application like a disposable well plate. The goal of our work has been, to make a full size DWP dispenser suitable to a standard 384 well plate by rapid prototyping in polymers. Because of the sophisticated microfluidic layout of one dispensing unit, which has to assure capillary filling of the system and a homogenous dispensing, we have chosen a prototype fabrication by micromilling. Micromilling has already been used before as a fast possibility for manufacturing of microfluidic structures [3]. In Fig. 1 a prototype of a 384 channel DWP chip made of a cyclic olefin copolymer (COC) by 3D micromilling is displayed.

System Description

The complete dispensing unit consists of a pneumatic actuation unit driving the DWP (fig. 2). The DWP dispensing chip itself consists of numerous dispensing elements arranged very closely at conventional well plate spacing. The array of dispensing elements is surrounded by an unstructured border enabling better handling and sealing of the upper surface when the DWP chip is loaded into the actuation unit. The actuation unit together with the loaded DWP chip builds a small pressure chamber which is connected via a fast switching valve to a pressure accumulator. The operation pressure with inert nitrogen gas can be set between 0-50 kPa. The pressure chamber which has the footprint of the complete upper surface of the DWP (120mm x 80 mm) is designed with low volume, so that the operation pressure is reached within 4-6 milliseconds.

Fig. 1: *Photograph of a 384-unit DWP made of COC (TOPAS 5013/size 120x80 mm²; pitch: 4.5 mm).*

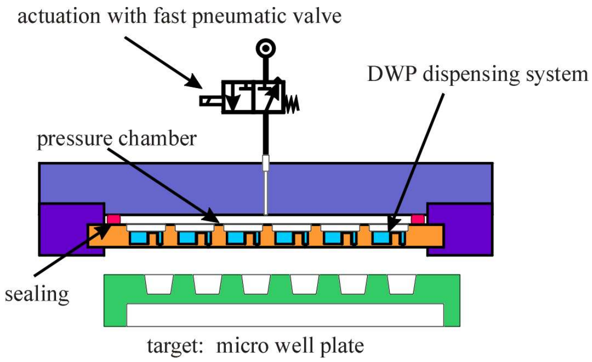


***Fig. 2:** schematic setup of DWP actuation unit*

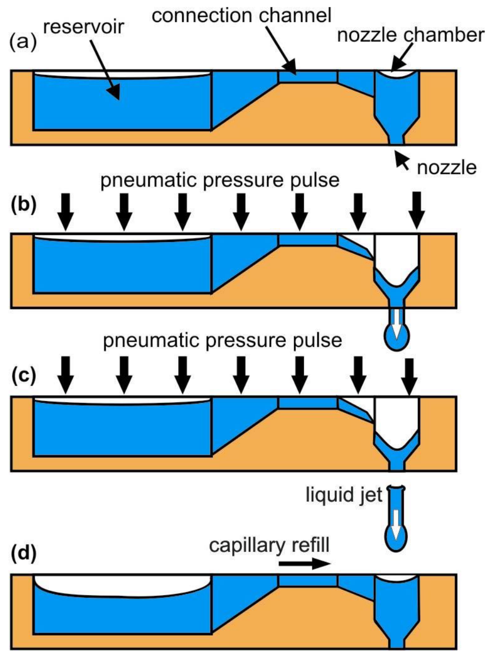


***Fig. 3:** DWP Dispensing process:*
- *a) basic elements*
- *b) Jet ejection by pneumatic actuation*
- *c) dispensed volume defined by volume of nozzle chamber*
- *d) refilling by capillary forces when pneumatic pressure turned off*

Each dispensing element of the DWP consists of three basic elements: a reservoir, a connection channel and a nozzle (cf. fig. 3a). For the experiments reported here we have chosen a pitch of 4,5 mm corresponding to the 384 -well plate format. When the reservoirs - which offer a volume of approx. 6 µl - are filled with liquid, capillary forces lead to self priming of the nozzles via the connection channels if the contact angle of the liquid related to the channel surface is below 90°. By applying the pressure pulse to the whole upper surface of the DWP the liquids contained in the nozzles (50 nL each) are driven out generating free liquid jets (fig. 3 b-c). Thus one pressure pulse empties all nozzles of the complete DWP simultaneously. Due to the pressure gradient which is negligible across the connection channels, and because of the flow resistance of the connection channel no considerable flow from the reservoirs occurs during that time. After switching off the driving pressure the nozzle chambers refill immediately again by capillary forces (fig. 3d).

Design for Micromilling

Earlier prototypes of the DWP done by reactive ion etching (RIE) in Silicon [1, 2], caused the presumption that the design of the nozzle can be optimised to reduce spraying effects. Simulations [4] have shown that optimum performance requires a real 3D-geometry including capillary channels in tapered nozzles which cannot be achieved with lithographic techniques.

Problems of the RIE/lithographic design like a geometrical diameter step of the nozzle causes a rest liquid volume in the corner of the step. This rest volume resides in the nozzle when the main volume is already dispensed and the jet tears off followed by a gas flow through the nozzle. The relatively fast gas flow partially drags out liquid of the rest volume resulting in a spray effect. A tapered nozzle reduces this effect. Therefore the nozzle has been designed as a two step drilling whole, with a conical diameter reduction from 300 µm to 100 µm at the nozzle orifice. The tapering has been achieved by using a drill with a lip angle of 90°

To guarantee the self priming of the nozzles via the connection channels, the geometry of the connection channel inlet, the channel itself and the transition to the nozzle is important. We have chosen for the channel cross section a tapered design which can be made by a tapered micro cutter. By arranging the cutter path as a 3-dimensional route from the liquid reservoir to the nozzle it was possible to overcome sharp edges in the channel geometry. Important for a proper fluidic self priming is the idea of continuous edge corners which guide the liquid meniscus through the connection channel. This idea can be transferred easily if the cutter creates one continuous 3-dimensional path during machining. It starts from the level of the reservoir base (depth 600 µm) going within a length of 500 µm to a reduced depth of 130µm. This depth is kept over the complete channel length of approx. 2mm. The transition to the nozzle is done by increasing the depth again for 200 µm until the nozzle centre is reached.

In figure 4 the fluidic part of such a design is depicted. It is visible that the channel base is build by a continuous plane with a width of approx.

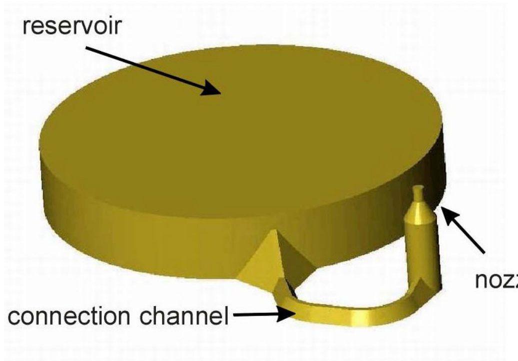
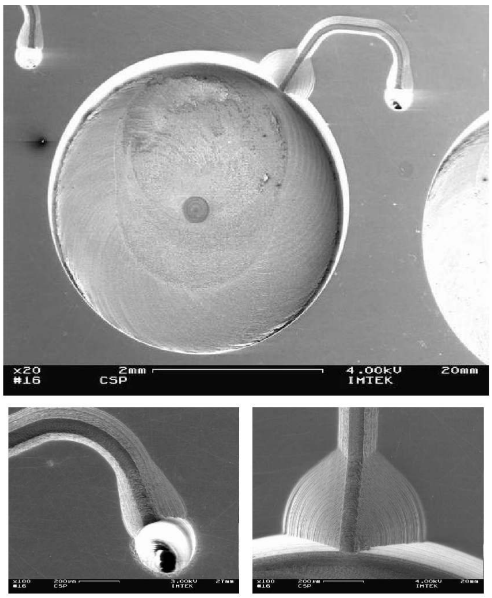


Fig. 4: *Fluidic part of the dispensing unit showing the real 3D geometry (upside down view)*

50 µm. The corners of this plane create high capillary forces guiding the meniscus while the upper area with a with of 200 µm creates comparably small fluidic resistance.

Fabrication Technology

To prove the feasibility of high-speed micromilling of real 3D geometries we realized DWP prototypes with 24 parallel dispensing units from PMMA and PEEK. In the final version we used injection moulded plates of the size 120 mm x 80 mm with a thickness of 1mm ± 20 µm made of COC (Topas



(a) (b) (c)

Fig. 5: *SEM picture of high-speed micromilled structures:*

a) dispensing unit
b) detail of the connection from nozzle chamber to supply channel
c) detail of the connection from reservoir to supply channel)

5013). On this plate 384 dispensing unit have been realized as shown in fig. 1. The plate was clamped for machining in a specially made holding fixture to achieve a low vertical deviation of the plate height.
First the reservoirs with a diameter of 3.6 mm have been machined. Afterwards the upper nozzle area with a tapered drill of 300 µm diameter was drilled with a speed of 8,000 rpm. Then the 3-dimensional path building the connection channel was micromilled with the tapered cutter.
In a final step the lower nozzle with the nozzle orifice of the dispensing device was drilled with a diameter of 100µm also at 8,000 rpm. Figure 5 shows SEM pictures of the high-speed micromilled structures:

- conical channels (upper / lower width: 200 µm / 50 µm) with a depth of 130 µm to maximize capillary pressure and minimize flow resistance (figure 5 a/c)
- tapered nozzles with upper / lower diameter of 300 µm / 100 µm (figure 5 b)

Milling speeds of 8,000 rpm result in a acceptable surface quality for PMMA and COC in this micro dimensions. It was observed that for PEEK the speed is to low and smearing of structure details occurs.

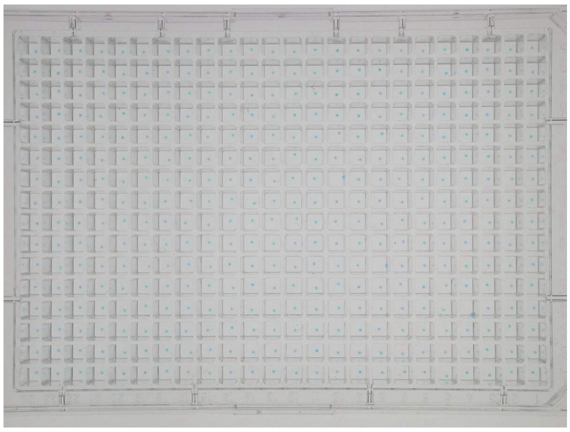


Fig.6: *Dispensed DMSO droplet array of the 384 DWP made by high-speed micromilling*

The nozzle side of the DWP chip has been modified afterwards by an ultra-hydrophobic coating to enhance the dispensing of liquids. Droplet ejection at the nozzle is much improved when having a non–wetting surface outside the nozzle orifice. This is especially visible when looking at the printed droplet array of the 384 format DWP (figure 6). The self priming of the micromilled DWP made of COC with DMSO (dimethylsulfoxide) is possible without any surface treatment. For water based solutions we used a primer treatment for surface modification in the connection channels and the inner nozzle area. Plasma treatments and gas phase

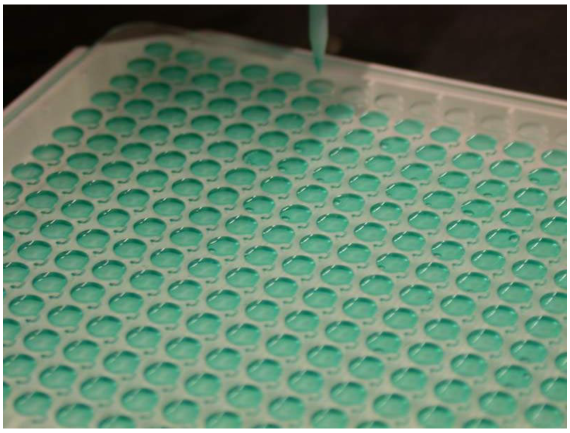


Fig.7: *Automated filling of the 384 DWP made in COC with aqueous buffer and self priming of the nozzles*

fluorination are possible alternatives for surface activation on an industrial level. The filling of the reservoirs of the 384 DWP chip with a conventional Liquid handling Automation and the self priming of the channels and nozzles is depicted in figure 7.

Results

We achieved fabrication tolerances of less than 5 µm in the nozzle area. This low variation results in an good dispensing performance of the DWP device. The mean dosage volume of all nozzles was determined to be 60 nL with a reproducibility taken by gravimetrical measurements with a Satorius NC2 balance of better than 5 % (figure 8).

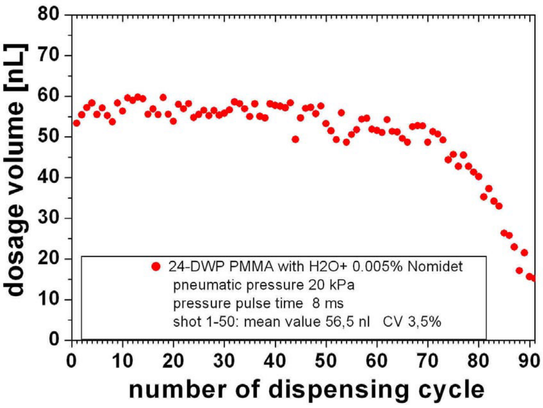


Fig.8: *Gravimetrical measurement of mean dosage volume of the 24-dispensing unit DWP in PMMA showing the reproducibility within* 90 *successive dispensing cycles*

Fluorescence measurements of a dispensed droplet array of buffer (H_2O with 0,5% Nomidet) labelled with Cy5 taken with a LaVision BioTe´s BioAnalyzer 4F/4S indicate a homogeneity of droplet volumes within the array of less than 11 %.

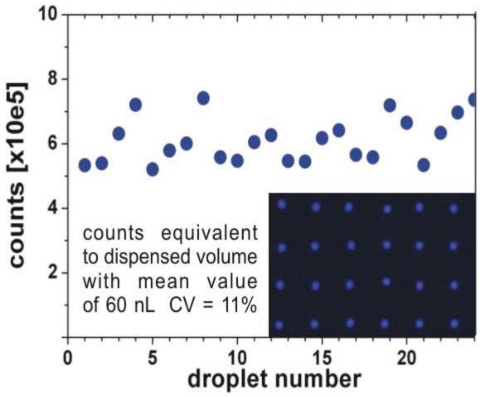


Fig.9: *Fluorescence image of dispensed droplet array (buffer labelled with Cy5) showing CV of 11 % at dispensing volume of 60 nL*

Conclusion

Real 3D microstructures with typical dimensions of 50 µm to 300 µm can be fabricated by high-speed micromilling having tolerances of less than 5 µm. Thus micromilling is an easy way for rapid prototyping of even complicated microfluidic structures in polymer materials. The basic structures presented in this paper also apply to other applications in microfluidics. The discussed fabrication technology therefore enables a significant extension of the design space. The example of the DWP demonstrates that it is possible to get within an array of 384 microfludic systems a good accuracy of the structures resulting in a good homogeneity of the dispensing quality.

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