Tailored microfluidic chips for biotechnology

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Abstract

This work presents new approaches for the manufacturing of tailored microfluidic bioreactor chips, adapted to the requirements of innovative methods and to the evolutionary state of the process design. Taking a microfluidic bioreactor chip for cell-free protein synthesis as an example, this study describes in detail the advantages and disadvantages of different manufacturing procedures with regard to cost efficiency and process-related functionality. In this connection, different production processes for the manufacturing of prototypes, small and medium batches and for the manufacturing of mass-products are compared. Moreover, processing parameters were optimized in terms of the technological relevant characteristics such as surface roughness, planarity or structural resolution, were made.

The qualitative assessment of the manufactured structures is made by manufacturing metrology and by an experimental study, based on the activity and yield of the cell-free expression of a fluorescent sample protein.

As a result of this work, a detailed procedure for the manufacturing of microfluidic chips is recommended, along with an assessment of the impact on the performance of a biological system in the microfluidic devices.

1 Motivation

Microfluidic chips are used for small scale biochemical reaction such as the cell-free synthesis of proteins. Several technologies exist to process structures on those chips. Here appropriate production-technological processes for plastic chips are evaluated and discussed. Considering the general process capability of the used manufacturing techniques, a comparison regarding structural resolution, dimensional accuracy, shape stability, quality of the machined surfaces and machining time is executed.

2 Evaluation of appropriate manufacturing methods

In particular, for the requirements of cell-free and medical biotechnology as small reaction volumes, excellent surface qualities and a high dimensional stability, there is a demand for microfluidic chips with microstructures having dimensions in the range of several millimeters down to 100 µm [1]. A simple example of the here used evaluation structure is shown in Figure 1.

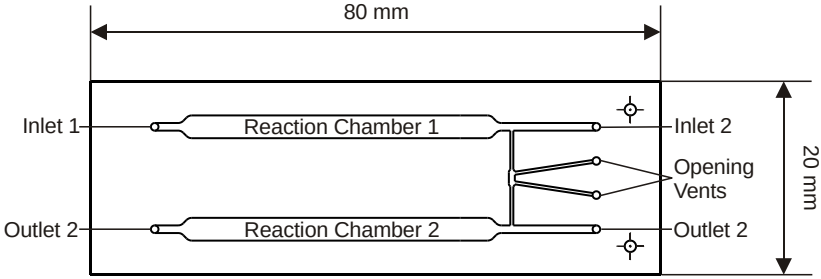

Figure 1: Schematic diagram of the evaluated structure

In pilot phases diverse structural variations are often needed [2]. Thus, a production chain that allows a fast evolutionary enhancement of the reactors is necessary. Laser ablation, direct micro milling and injection molding meet the demands to varying degrees and are employed for the manufacturing of microfluidic chips (Figure 2).

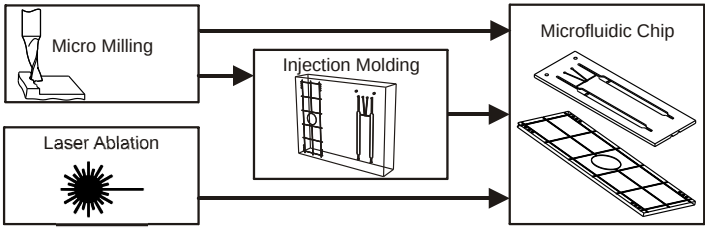

Figure 2: Schematic diagram of the evaluated process chain

The manufacturing of microfluidic chips by *laserablation* was done with a picosecond-laser-source with a mean power of 5 W, a pulse-duration of 15 ps and a repetition rate of 200 kHz. The positioning was done with an yx-Laserscanner and the ultra-precision machine tool Kugler MicroGantry Nano 5x. The structural resolution is limited by the focusing optic, beam quality, pulse length and repetition rate. The minimum producible structure size is 5 µm in which a feasible corner radius is 3 µm. The *Micro-Milling* was carried out using micro-cutting tools with a diameter between

50 µm and several millimeters and the machine tool Wissner Gamma 303. The minimum structure size is in the range of 50 µm and the minimum corner radius correlates to the half of the tools diameter. *Injection molding* was done with a Battenfeld EM500 injection molding machine. The resolution capacity of the process is directly depending on the injection molding tool. The manufacturing procedures, used for the production of the mold must therefore have equal or higher reproduction accuracy than the desired microfluidic chips. In this case study, replication was done with Topas 6013M-07.

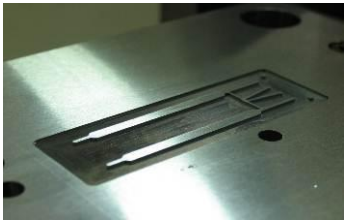

Figure 3: Mould insert milled in tool steel for the replication of the reference structure by injection moulding

3 Machining results

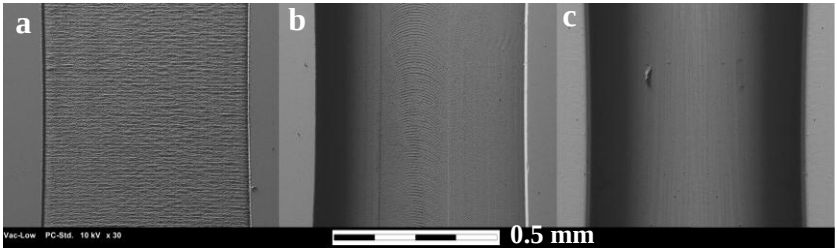

Figure 4: SEM image of a laser-machined Channel (a), a micro-milled channel (b) and an injection molded channel (c)

In order to evaluate surface qualities as an example for the whole study, each of the manufacturing procedures was evaluated. Clear differences of surface qualities are obvious (Figure 4). The laser machined surfaces show a reflection of the laser pulses used for the processing (Figure 4a). In Table 1, the achieved results are summarized.

Table 1: Procedure comparison of the evaluated manufacturing processes in compliance with the suitability for the manufacturing of microfluidic chips

Technology	Minimum feature size	Aspect ratio	Lifetime	Processing cost	Machining time
Laser ablation	++	o	-	++	+
Micro-milling	+	+	o	o	++
Injection-molding	++	o	++	--	-

++ very good, + good, o neutral, - poor, -- very poor

Furthermore its surface roughness is in the range of Ra = 0.8 µm. In contrast, the micro-milled surface shows significantly less machining marks and a surface roughness of Ra = 0.2 µm (Figure 4b). The injection molded microfluidic chips have a substantially reduced surface roughness of Ra = 14 nm (Figure 4 c).

4 Biotechnological evaluation

The biotechnological evaluation of the manufactured microfluidic chips was done by the synthesis of a green-fluorescent model protein (GFP). The microfluidic channels were filled with a solution containing all reagents needed for a cell-free synthesis reaction of GFP using a bacterial cell lysate. After one hour incubation at 37 °C, a qualitative analysis was carried out by fluorescent microscopy, furthermore the synthesized protein was visualized in a protein gel (Figure 5). Using an electrophoretic technique, proteins in a solution can be separated according to their weight. The investigation demonstrated a comparable rate of synthesis for all structures examined (Figure 5a). However, in the laser machined channels agglomeration effects may be of influence be evident (Figure 5b).

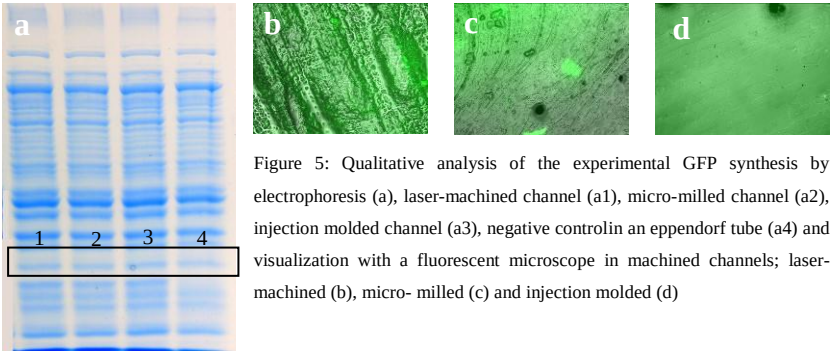

Figure 5: Qualitative analysis of the experimental GFP synthesis by electrophoresis (a), laser-machined channel (a1), micro-milled channel (a2), injection molded channel (a3), negative controlin an eppendorf tube (a4) and visualization with a fluorescent microscope in machined channels; laser-machined (b), micro- milled (c) and injection molded (d)

5 Outlook and Conclusion

Surface roughness, as a key parameter for the manufacturing of microfluidic chips, was presented in this case study as an example. The remaining parameters (Table 1) are under final investigation. The entire study will give a detailed and process based recommendation for the manufacturing of microfluidic chips.

References:

[1] Rötting, O.; Röpke W.; Becker, H.; Gärtner C.: Polymer microfabrication technologies. Microsystem Technologies 8, S. 32 – 36, 2002