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Manufacturing, replication and assessment of microfluidics for blood plasma separation

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Abstract
Point of care diagnostics gain importance with regard to novel diagnostic techniques. Future applications, for example based on cell-free DNA, are prenatal or cancer diagnostics. With regard to these methods a large influence of natural blood degradation during full blood storage and transport before laboratory based separation restricts possible applications. With a point of care plasma separation device, the plasma can be separated from full blood directly after blood collection. Thus, diagnostic markers remain undamaged and allow a highly specific detection.
Targeting microfluidics for point-of-care blood plasma separation, this work presents the development of a microfluidic blood plasma separation device along a novel process chain. Besides system design and manufacturing technologies for prototypes, the development of technologies for replication, closure of microfluidics and affecting of surface properties are under the special scope of this investigation.

Keywords: microfluidics, point-of-care diagnostics, plasma separation, micro-replication

1. Introduction

The basic idea of point of care blood plasma separation is the immediate treatment of full blood right after sample collection. Due to cell lysis and degradation effects during storage and transport, analysability of blood plasma decreases [1]. Especially, the rapid partition of cellular components is essential for novel diagnostic methods based on cell free DNA (cfDNA). Standard blood plasma separation requires centrifuges and handling time. Physicians normally avoid extra investments and this dramatically reduces the market potential for this non-invasive and innovative diagnostic tool. Thus a fast and easy microfluidic disposable blood plasma device would be an opportunity to gain of high quality plasma and to support new diagnostic methods.

2. Framework conditions for microfluidic blood plasma separation

Microfluidic blood plasma separation in laboratory scale has been presented by several groups [2]. However, novel diagnostic methods analysing cf DNA need a certain volume of up to 1.5 ml (Table 1) in an acceptable time that could not be provided by microfluidic blood plasma separators yet. To obtain full functionality of analytical systems, the complete separation of cellular components is necessary.

Table 1. Needed plasma volumes for molecular genetic analysis.

Application	Plasma volume min. in ml	Separation time in min
Point of care cfDNA detection	1.5	≤ 5
Lab-on-a chip rtPCR diagnosis	0.5	≤ 1
Lab-on-chip diagnosis with specific markers	0.05	≤ 0.2

2.1. Fluid mechanical principle

The general basis of this work is a microfluidic unit presented by Demulliez at al. [3]. The mode of action is based on a constriction of a microfluidic channel and an increase of pressure and velocity. As a consequence, cellular components are collected in the centre of the flow. A sudden expansion of the fluidic channel leads to the formation of a cell free area (Figure 1). A plasma channel connected in this area derives the plasma. Geometrical parameters of the microfluidic elementary cell are presented in Figure 2. In accordance to a specific inlet flow a complete separation cellular components can be achieved.

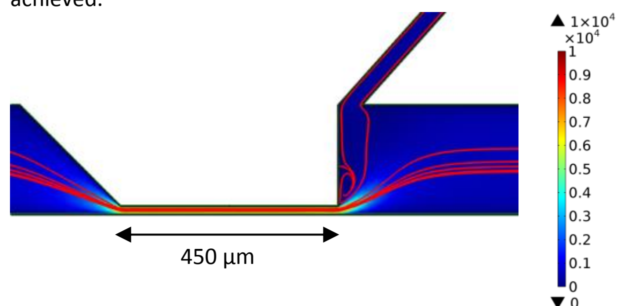

Figure 1. Flow simulation of microfluidic elementary cell with representation of flow paths created with Comsol Multiphysics.

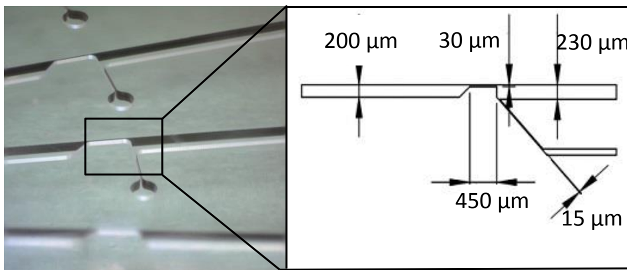

Figure 2. Geometrical parameters of the microfluidic elementary cell.

2.2. Upscaling of microfluidics

In order to achieve an acceptable volume/separation time (Table 1), 42 single separation structures had to be parallelized. Due to fluidic interaction of the structures and pressure losses on small channels, a series connection is not applicable. A parallel connection of elementary cells in a ring shape was used to ensure a uniform incident flow to the entirety of the structures.

3. Assembly concept

The disposable microfluidic device (Figure 4) comprises of three components. A flexible middle layer with the microfluidic structures is embedded between two cover structures. Simultaneously, these structures fulfil interface and closure functions. Due to the layout as a disposable system, manufacturing technologies for mass production are indispensable. Therefore, the components are manufactured by injection moulding. Cover structures were manufactured of thermoplastic material COC Topas 6013, Topas Chemicals, Germany. The microstructured middle layer was manufactured of Liquid Silicone Rubber (LSR) MS1003 provided by Dow Corning Corp., US.

4. Manufacturing of master structures and replication

The experimental evaluation of single and parallelized structures showed a dependency of separation efficiency on fluid flow and channel quality. A maximum surface roughness of Ra ≤ 20 nm is necessary. Moreover, geometrical features as channel width and depth must be manufactured with narrow tolerances. For a process reliable replication of the obligatory structures, master structures and mould inserts had to be performed by ultra-precision machining with monocrystalline diamond tools (Figure 3).

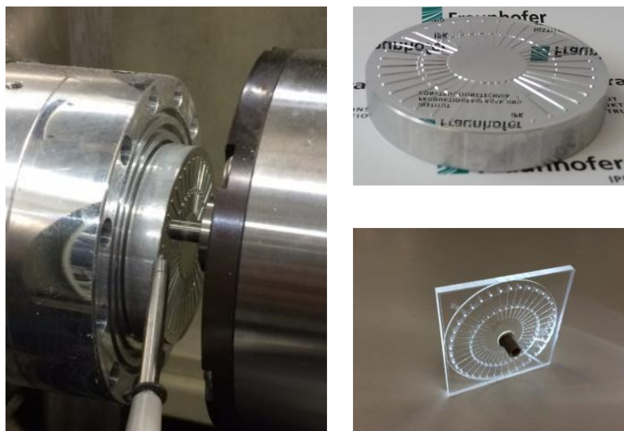

Figure 3. Ultraprecision machining of microfluidic mould insert (left), finished mould insert (upper right) and replicated and assembled blood plasma separator (lower right).

The Injection moulding of the sealing structures was performed with an Arburg Allrounder 370 machine tool. The manufacturing of the middle layer was performed with a Babyplast LSR micro-injection moulding machine tool provided by Christman Kunststofftechnik GmbH, Germany.

5. Fluidical and biotechnological evaluation

The parallelized system was operated using a commercial syringe pump (Figure 4). The single structure inlet volume flow of $\dot{V}_{in}$ = 80 µl/min was nearly adjusted linearly and a full inlet volume flow of $\dot{V}_{in,full}$ = 2,80 ml/min maintained. The full functionality of the parallelized structures could be achieved.

Figure 4. Fully assembled microfluidic blood plasma separator (left) and blood plasma separation (right).

Blood plasma samples were analysed and compared with standard plasma separation technique by centrifugation. Comparable amplification results of cf DNA (Figure 5) in both samples demonstrates the technical feasibility of microfluidic blood plasma separation and provides a next step towards the use of cf DNA derived of blood plasma as an routine diagnostic sample.

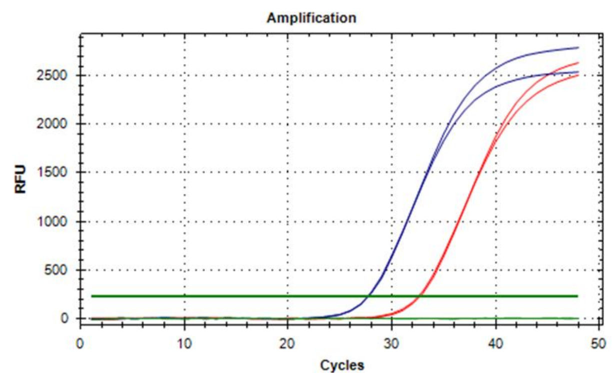

Figure 5. Amplification of human cell free DNA (oestrogen receptor 1) from a plasma sample separated by microfluidic blood plasma separator (red) and from a conventionally separated blood plasma sample (centrifugation) (blue).

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