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Injection molded pinched flow fractionation device for enrichment of somatic cells in cow milk

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ABSTRACT

In this paper the continuous microfluidic separation technique pinched flow fractionation is applied to the enrichment of somatic cells from cow milk. Somatic cells were separated from the smallest fat particles and proteins thus better imaging and analysis of the cells can be achieved. The enrichment was performed using an all-polymer pinched flow fractionation device fabricated by injection molding. The polymer chips were bonded to a 500 μm polymer foil using UV assisted thermal bonding. The quality of the final devices was reproducible and the injection molding process combined with the use of cheap materials ensures the possibility for device mass production.

1. Introduction

Dairy production is a large industry and the milk quality depends strongly on the animal health. Milk quality is measured by the concentration of somatic cells, which are cells from the animal that naturally are found in the milk. In the EU, milk is discarded if the number of somatic cells is above 400,000 mL^{-1} [1]. The majority of the somatic cells found in milk are white blood cells, which can be divided into several types. The quantity of white blood cells increases and the cell type distribution changes in response to an animal getting an infection. Other somatic cells found in milk are less important to the animal health. To test the milk quality dairy farmers use commercial cell counters where nuclear DNA is stained, and fluorescence detection is used to estimate the cell count. This method only allows to assess cell counts corresponding to milk quality below standards. Measurements on not just the amount of somatic cells, but also the percentage of each type would instead provide dairy farmers with valuable information to prevent milk waste. Optical imaging of somatic cells could allow such early diagnostics of animal infections. However, optical detection of cell types is very difficult because of the blur and contrast reduction induced by fat particles and proteins found in milk samples. There is therefore an interest in a method for enrichment of somatic cells in milk to allow for in-line optical characterization of the white blood cell distribution. This can for example be done by separating fat cells and proteins from the somatic cells. Fat particles vary in size from 1 μm to 15 μm, with the highest occurrence around 4 μm [2], and proteins are even smaller. Most white blood cells have sizes between 8.5 μm and 10 μm [3]. This size difference can be exploited by lab-on-a-chip (LOC) based separation techniques.

In recent years the interest in LOC systems for cell handling has increased. LOC devices can be produced at a low cost and enable label-free detection to be combined with single cell handling, which can be important when investigating cell biology. Several microfluidic techniques have been developed for cell separation and sorting [4] including deterministic lateral displacement [5], hydrophoretic separation [6], use of hydrodynamic lift forces [7] and pinched flow fractionation (PFF) [8]. These methods have for example been used for enrichment of circulating tumor cells [5] and separation of platelets and red blood cells [6,7]. Other LOC techniques for separation relying on applied external fields have been developed. These include, among others, dielectrophoresis [9], magnetophoresis [10], acoustophoresis [11] and centrifugal disk platforms [12], but are not relevant for somatic cell separation because of increased sample preparation time and running costs compared to the method described in this paper.

LOC microfluidic separation devices are often operated by applying a pressure-driven flow and use either a passive or active control of the cut off size. Passive devices are attractive as they require no other actuation than a source of pressure or vacuum.

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However they have the disadvantage that they cannot be adjusted to different samples because the cut off value is fixed by the device geometry. Recently, a flow displacement device was fabricated where the cut off value is adjusted by deforming the device made of elastomeric PDMS [13]. The devices presented in this paper use the pinched flow fractionation principle. In this size separation device, the cut off size can be adjusted by varying the pressures applied to the drain outlet, thus the separation can be adjusted to different samples. Furthermore, PFF devices are passive microfluidic devices that can be mass produced using the plastic industry standard method injection molding.

PFF is a continuous microfluidic size separation method. A sample containing particles of different sizes is injected into a microfluidic system and enters a narrow channel called the pinched segment, where the particles are aligned against the channel wall because of high flow from a buffer fluid, see Fig. 1a. Next the aligned particles enter a broader channel where they follow a streamline according to their center of mass. In the broader segment the particles of different radii (r and R in Fig. 1a) are positioned at different distances to the wall (dr and dR respectively) thus they can be collected in different outlets. In the fabricated devices there are two outlets for separation of the sample, so that particles bigger than a critical radius r_c are separated from smaller particles.

PFF was first demonstrated in 2004 [8] and has been used extensively for size separation of polymer beads from the microscopic to the sub-micrometer range. The designs have become increasingly advanced using different geometries with several separation outlets or microvalves to precisely control the flow rates [14,15]. It has also been shown that PFF can be used for sorting of non-solids such as oil emulsions [16] and it has been used for a few applications for diagnostics, such as detection of single nucleotide polymorphisms for fast genotyping [17] and separation of red blood cells and white blood cells [18].

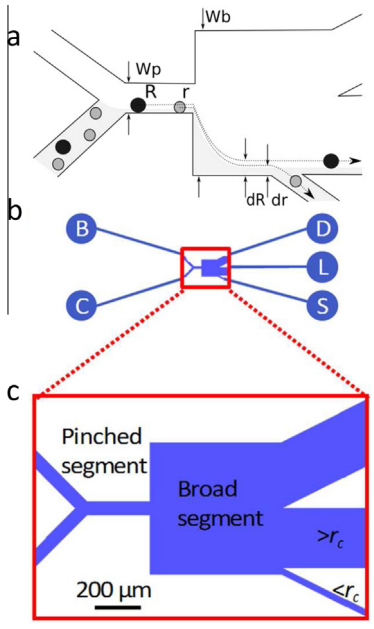

Fig. 1. (a) Sketch of the PFF principle, where two particles of different radii are aligned in the pinched segment and separated into the outlet for small and large particles respectively. (b) The inlets and outlets on the PFF devices. Inlet C is for the cell sample, inlet B is for the buffer, outlet S (L) is for collecting small (large) cells with a radius below (above) the critical radius r_c, and outlet D is a drain for collection of the buffer. (c) Zoom in at the pinched and broad segment. The height of all channels is 30 µm. The width of the pinched segment W_p is 100 µm and the width of the broad segment W_b is 1000 µm.

In this paper we demonstrate that PFF devices that can be cheaply mass produced by injection molding can be used to enrich somatic cells from milk by separating them from the smallest fat particles and proteins, thus enabling optical characterization of the somatic cells.

2. Materials and methods

2.1. Chip design

A sketch of the channel design is shown in Fig. 1b–c. The PFF device has two inlets, a pinched and broad segment and three outlets. The first two outlets are for separating the sample into particles smaller and larger than r_c and the third is used as a drain for most of the buffer solution injected from inlet B in order to pinch the sample in the pinched segment. The height of all channels is 30 µm such that particles as big as 25 µm can be handled.

2.2. Fabrication

A nickel shim for injection molding of the chips was fabricated using standard clean room processes [19,20]. A four inch silicon wafer was treated with hydrofluoric acid and coated with positive photo resist for I-line photolithography. The resist was developed creating an etch-mask. The silicon wafer was etched 30 µm using deep reactive-ion etching and the leftover resist was removed by plasma ashing and acetone. A nickel seed layer was sputtered onto the wafer thus a 30 µm thick nickel layer could be electroplated. Finally the silicon was removed using a KOH etch leaving only the nickel shim. The final shim was imaged in a scanning electron microscope (SEM) before injection molding, see Fig. 2a.

The microchannels were injection molded in the polymer TOPAS 5013 using a tool temperature of 140 °C and a cooling time of 90 s. A chip was imaged in a SEM after injection molding, see Fig. 2b.

2.3. Thermal bonding

The channels were sealed with a 500 µm thick TOPAS 5013 foil using UV assisted bonding. The chips were bonded at 120 °C and a pressure of 51 bar for 5 min using a P/O/Weber press. Prior to bonding the polymer device and lid had been exposed to UV light from a mercury arc lamp for 30 s. A similar bonding process has previously been shown to yield a high strength sealing, meaning the chips can withstand high applied pressures [21]. An image of a bonded chip is shown in Fig. 2c. Due to the molding parameters, the top edge of the microchannels is rounded, causing the bonding of the foil to be incomplete at the edge of the channel. The resulting shallow gap around the channels is visible on the optical images (Fig. 2c), however this is not expected to affect the fluid flow.

2.4. Sample preparation

Fluorescent beads were used to test the PFF device and to set r_c before experiments with milk. Two bead sizes were used: Green fluorescent (468/508) 5 µm polystyrene beads from Duke Scientific and orange fluorescent (540/560) 10 µm FluoSpheres® polystyrene beads from Invitrogen. The beads were mixed with 0.1% Triton X-100 in Milli-Q water to a concentration of 300,000 mL^{-1}. Cow milk supplied from a dairy farmer was mixed with 0.1% Triton X-100 in Milli-Q water to concentrations between 5 vol% and 10 vol%. Milk samples were kept in a refrigerator before use and were no more than two days old. The concentration of somatic cells was measured before and after experiments using a DeLaval cell counter (DCC) [22]. The DCC works by sucking approx. 60 µL