SEPARATION OF SPERMATOZOA WITH A COMBINATION OF PINCHED FLOW FRACTIONATION AND TANGENTIAL FILTRATION

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ABSTRACT

We demonstrate a pinched flow tangential filtration method to sort spermatozoa from larger particles with a spermatozoa collection efficiency of 94±2% and a separation efficiency of 100%. In conventional pinched flow fractionation (PFF), an observed tumbling-like rotation of spermatozoa complicates their separation from other particles. The new design circumvents this effect.

KEYWORDS: Microfluidic Sorting, Spermatozoa, Pinched Flow Fractionation, Tangential Filtration

INTRODUCTION

In men suffering from non-obstructive azoospermia, production and development of spermatozoa is greatly reduced. Spermatozoa are obtained via a biopsy, from which the spermatozoa are separated manually from the 10-20µm sized other cells. This is an expensive and time consuming process, which cannot always be completed before the waiting oocyte dies [1]. Here we offer an automated procedure.

RESULTS AND DISCUSSION

Classical PFF design

PFF is a popular way to separate particles with a high separation resolution and minimal risk of clogging. Separating spermatozoa with PFF poses two challenges: the small head size (4µm) and oblong shape (50x4µm). Small size necessitates a small focus width (width of the focused particle flow) [2]. Due to the parabolic flow profile however, small focus widths require very high flow rate ratios (Figure 1). Alternatively a small channel width can be used, but the minimum channel dimensions are determined by the diameter of the largest particle (~50µm).

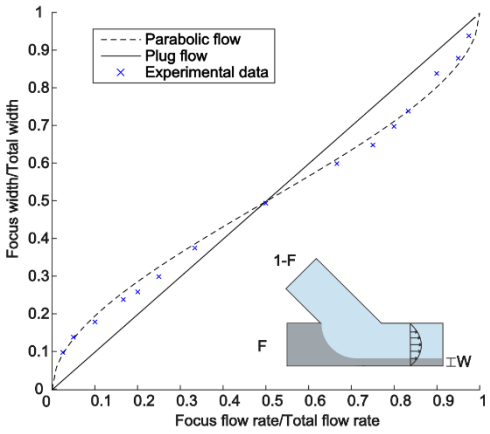

Figure 1: Width of the particle flow (W) as function of the flow rate ratio (F).

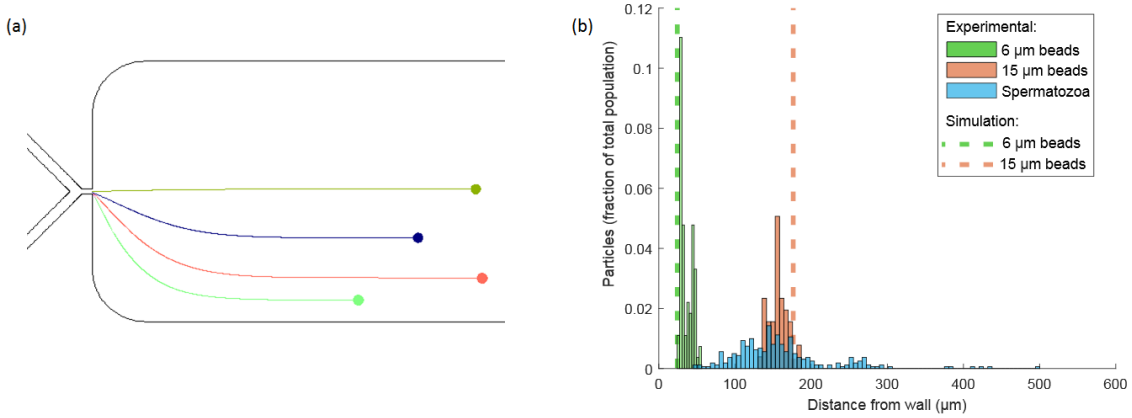

Figure 2: (a) Simulation of the 6, 15 30 and 45µm bead trajectories in the PFF device (COMSOL 5.1). Pinched region is 50µm. Theoretical separation distance of 6 and 15µm beads is 150µm. (b) Particle positions in the broadened segment. 6 and 15 µm beads are well separated as average separation distance is 125 µm (simulated 150 µm). The spermatozoa form a broad band over a distance of more than 250µm. Concentrations in inlet sample were $20{\cdot}10^6$, $20{\cdot}10^6$ and $5{\cdot}10^6$ per mL respectively for the 6 and 15µm beads and spermatozoa.

The oblong shape of the spermatozoa presents a further challenge, because they behave like a particle with any diameter in between the length of their major and minor axis, depending on their orientation with respect to the flow, and tumble in the parabolic flow. We confirmed this experimentally by polystyrene bead and spermatozoa separation and by COMSOL simulation for bead positions (Figure 2). Separation of sperm cells from beads (and hence normal cells) by PFF thus proved impossible.

Improved PFF/tangential filter design

In the literature, PFF has been combined with a hydrodynamic filter [3] or a micro-membrane [4]. Our improved design (Figure 3a) combines steric and hydrodynamic filtration to increase the collection efficiency (defined as obtained spermatozoa/total spermatozoa population) and separation efficiency while enabling visual inspection due to use of a pillar array as a sideways filter. In the pinched region of this device, the focused flow runs over a pillar array with 4µm spacing, which allows passage of the spermatozoa but prevents passage of the beads (Figure 3b). With this geometry, it is possible to force 100% of the spermatozoa-containing solution through the filter, while objects bigger than the pillar spacing continue along the main channel. A pulsatile flow was applied to prevent any residual fouling. A collection efficiency for spermatozoa of 91±1% (n=3) and 94±2% (n=3) with 100% purity was obtained with flow rate ratios of 1:4 and 1:16 respectively. 80,000 particles were processed per experiment in the device. For these proof of concept experiments, the samples contained a 1:1 mixture of beads and spermatozoa, a larger relative concentration of spermatozoa than in biological samples, to obtain reliable cell counts. In the near future, the device will be used in experiments with samples containing spermatozoa and cell types normally found in biopsies.

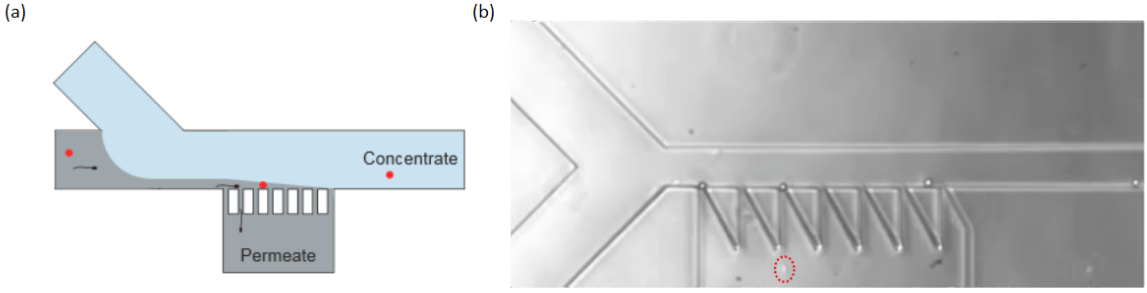

Figure 4: (a) Schematic representation of the improved design, containing a pillar array with a spacing of 4µm after a flow focusing region. Spermatozoa are able to pass the filter and end up in the permeate, while beads end up in the concentrate. (b) Spermatozoa (circled red) are able to pass the filter and end up in the permeate, while 11 µm beads end up in the concentrate.

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