SPERM CELL SEPARATION USING A SPIRAL CHANNEL

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ABSTRACT

This work presents a specifically designed spiral type inertial micro-fluidic channel for separating non-motile sperm cell from cellular debris. Demonstration with a mixture of non-motile sperm and red blood cells showed that 82% of sperm could be focused into a separate outlet from the red blood cells.

KEYWORDS: Sperm separation, inertial micro fluidics, non-motile sperm, mTESE, NOA

INTRODUCTION

Microfluidics devices for sperm manipulation have been of interest for several years and devices showing the sorting of healthy sperm for *In Vitro* Fertilization (IVF) have been demonstrated[1]. This technology enables automation to replace tedious, manual approaches to viable sperm cell sorting from semen specimens.

Until now, sperm separation with microfluidics has been based entirely on sperm motility [1]. Motility-based sperm separation technology will not work when viable non-motile sperm need to be separated in certain clinical specimens [1,2], such as for use in intracytoplasmic sperm injection (ICSI). Such specimens are typically acquired by micro-dissected testicular sperm extraction (mTESE), a procedure that requires separation of non-motile sperm, because testicular sperm aren't yet fully mature and generally have not gained motility. The specimen also contains a combination of red blood cells (RBC), white blood cells, sertoli cells and debris that must be distinguished from spermatocytes [2].

Here, we demonstrate the use of inertial microfluidic technology to separate sperm by focusing flow [3]. The separation method is label-free and unlike other work on microfluidic sperm separation, is not based on sperm motility. Results show the separation of non-motile sperm from a mixed sample containing non-motile sperm and RBCs.

STRUCTURE DESIGN / FABRICATION

Key principles of spiral channel separations were considered to define specific dimensions for separating non-motile sperm, include the ratio of inertial lift and Dean drag (R_f), the ratio of particle and channel dimension(λ) and the aspect ratio of the channel cross-section. When R_f, λ, and the aspect ratio (width:height) were >~0.08, >0.07 and 3:1, theory suggested that the sperm could be focused and separated from RBCs [3]. For calculation convenience, 5 μm and 10 μm diameter sphere particles were considered to represent sperm cells and RBCs accordingly and different flow rates were also considered with a range of 0.1ml/min to 1ml/min. Based on these calculations, the spiral channel needed to have specific values of width, height, turns, gap, and spiral radius, and those were 150 μm, 50 μm, 5 turns, 310 μm, and 0.9 cm respectively. The device was molded using SU-8 (SU-8 3000, MICRO CHEM) and PDMS (Sylgard 184, Dow Corning).

EXPERIMENTAL

The experimental system consisted of two syringe pumps, five 1 ml syringes and one micro spiral channel device (Fig. 1). One syringe pump injected sample through the inlet of the channel, and the other syringe pump pulled from the four outlets. The overall injection and removal flow rates were kept the same to get equal amount of sample from the outlets. The rate of injecting and pulling ranged from 0.1-0.3 ml/min with 0.05 ml/min step increases in rate for the various experiments.

The processed sample contained 2 million/ml of sperm and 5.3 million/ml of RBC in 1 ml volume. The original sample was diluted for some later experiments to avoid interference between the different particles that led to sample dispersion and poor results [3]. After running the sample through the spiral

channel, the samples were collected from different outlets (most outer outlet 1, outer outlet 2, inner outlet 3, most inner outlet 4), and were inspected using microscopy.

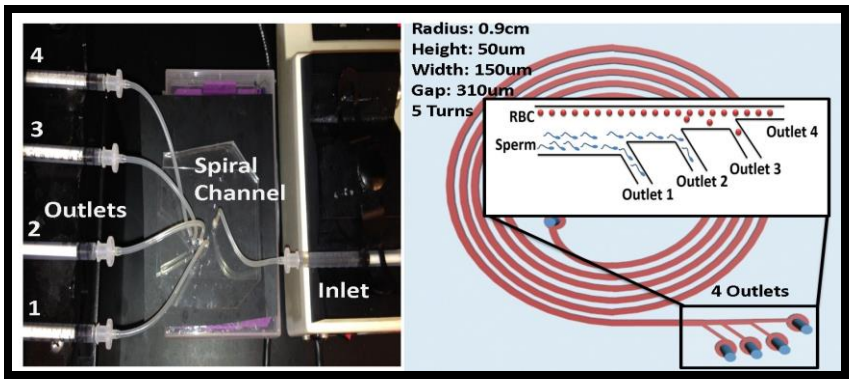

Figure 1: Experiment setup photo(Left), designed spiral channel(Right).

RESULTS AND DISCUSSION

The concentration of samples collected from outlets 1~4 were evaluated using a counting chamber under a microscope (20X).

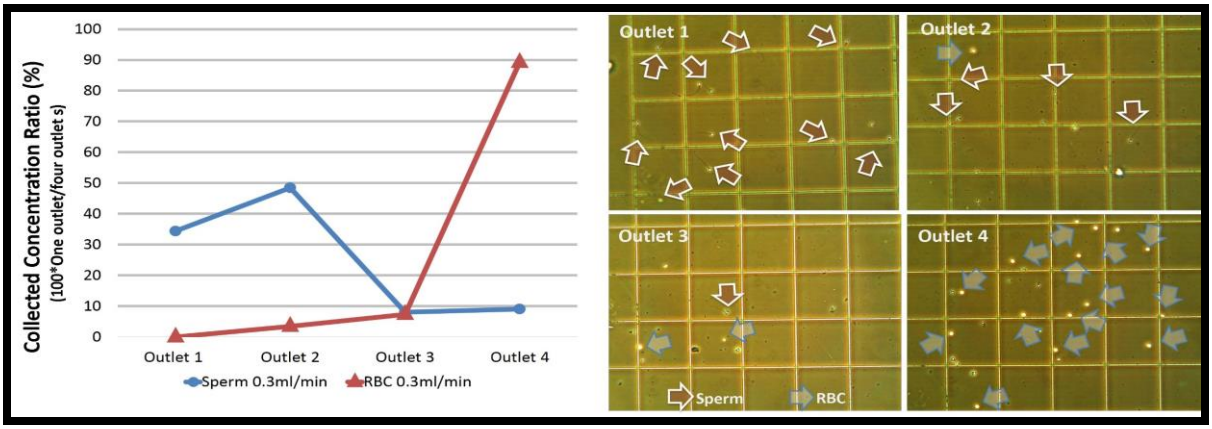

Figure 2: Mixed sample separation ratio(at 0.3ml/min) at each outlet(Left) Example photos (Right).

The concentration of sperm was clearly higher at outlet 1 and outlet 2 than at outlet 3 and outlet 4 with measurements of 1.7, 2.4, 0.4 and 0.45 million/ml respectively. In terms of concentration ratio the overall collected sperm at outlet 1~4 were 34, 48, 8, and 9 %, respectively. In contrast, the concentration of RBCs was clearly higher at outlet 3 and outlet 4 than at outlet 1 and outlet 2 with measured concentrations of 0.85, 10.3, 0, 0.4 million/min respectively. In terms of the concentration ratio of the overall collected RBC at outlet 1~4, those were 0, 3.4, 7.3, 89.2 % respectively. As expected relatively smaller cells mostly collected at the outer outlets (sperm cell, 82 %) and larger cells mostly collected at the inner outlets (RBC, 96 %).

CONCLUSION

Sperm and RBCs were successfully separated from each other in a microfluidic spiral device with over 96% of RBCs removed from the sample and 82% of the non-motile sperm recovered, suggesting that this technique might be useful for collecting small numbers of sperm from mTESE samples.

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