HUMAN SEMEN SORTING BY MICROFLUIDIC SYSTEM FOR OLIGOZOOSPERMIA CONCENTRATIONS AND VIABILITY ENHANCEMENT

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This paper reports a microfluidic system which can continuously sort motile sperms from semen sample based on sheath flow and the motility of sperms. Besides, we also dilute the normal semen samples to imitate the oligozoospermia patient for the viability enhancement and sorting efficiency by using the developed microfluidic chip.

The fabrication process for the microfluidic chip is similar to our previous work [1]. Our new method to connect the inlets and the syringe tubes is placing plastic tubes on the mold before PDMS formation, shown as Fig.1, which can prevent poisoning the sperms by using AB glue as the old method. This paper presents a method by using laminar flow sorting sperms in lower concentration of patients continuously.

The World Health Organization (WHO) had published the standard of normal human semen[2], including the sperm concentration as below:

- Mild oligozoospermia (2×10^7~1×10^7 sperms/ml);
- Moderate oligozoospermia (1×10^7~5×10^6 sperms/ml);
- Severe oligozoospermia (5×10^6~ sperms/ml).

Males who suffered from oligozoospermia are possible to be infertile; hence these people may need IVF or ICSI for assistance. For these procedures, a certain number of motile sperms are required, this research provides a simple and inexpensive method to accumulate motile sperms and enhance the viability of semen.

The fabrication process of microfluidic chip includes SU8 thick-film photolithography and soft-lithography by using PDMS (Polydimethylsiloxanes), shown as Fig.1. Inside the chip, laminar flows are generated by syringe pumps as two semen flows on the sides and one buffer flow in the middle. After flowing through the main channel, the dead sperms remain in the semen flow heading to the waste reservoir, and parts of the motile sperms swim through the boundaries to the sorting reservoir. Fig.2 and Fig.3 indicate the structure of the microchannel and flow rates simulated by ANSYS. Fig.4 shows how the motile sperms are sorted.

In order to identify our results, we define sorting efficiency as:

$$\text{sorting efficiency}(\%) = \frac{\text{live sperms in sorting reservoir}}{\text{live sperms in sorting} + \text{waste reservoir}} \times 100\%$$

Which means the percentage of live sperms swimming through the boundaries and being sorted in the sorting reservoir.

We do several times of experiments for each level of oligozoospermia samples, and take averages of each group. In Fig.5, we can see that before sorting, the control viabilities are in range of 60~70%; after sorting in the microfluidic chip for 1 hour, the viabilities increase to about 70~80%. The average viability enhancements are all more than 10% for these three levels. The sorting efficiencies are 12% or more, which means that there are 12% of live sperms collected from the original samples. These results show that our chip can separate live sperms from oligozoospermia samples successfully, and not only enhance the viabilities, but also increase the number of live sperms.

Word Count: 451

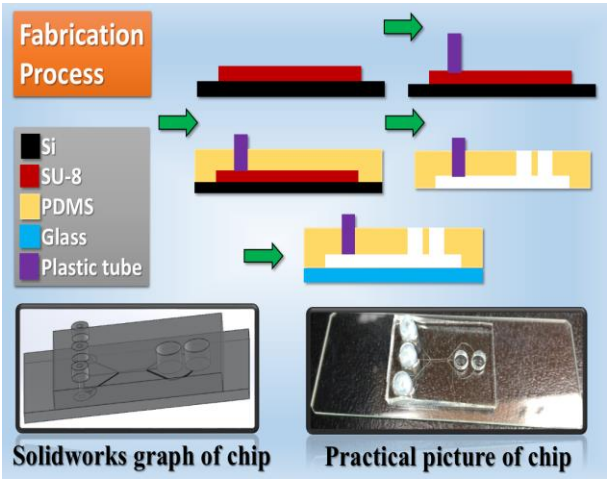

Fig.1. The fabrication procedures of the sperm sorting chip. Use SU-8 lithography process to make the mold and transfer to PDMS substrate, finally bonding with slides by oxygen plasma.

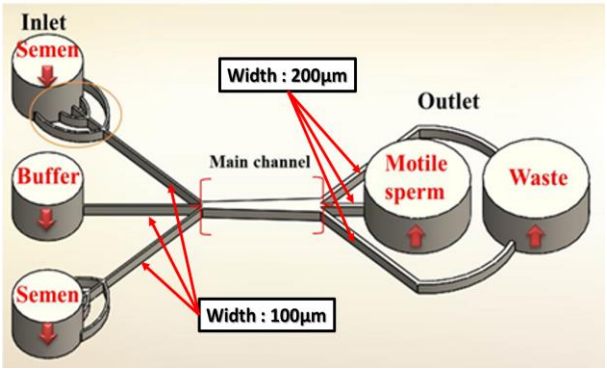

Fig. 2 The microchannel is designed as three inlets and three outlets. Inlet width: 100μm each; outlet width: 200μm each; height: 100μm.

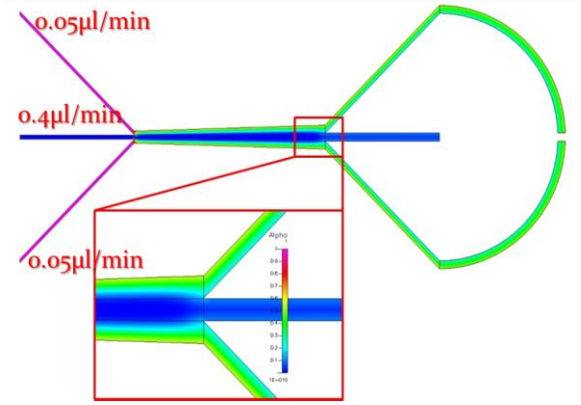

Fig. 3. Use Ansys simulation to determine the flow rates as semen flow(purple and green) 0.05μl/min and buffer flow(blue) 0.4μl/min, this ratio can prevent dead sperms rush into sorting reservoir.

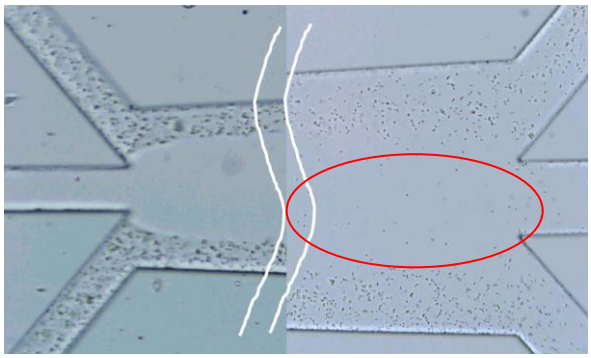

Fig. 4. Semen flow in the microchannel; left: inlets, right: outlets; the circle region is motile sperms swimming through the boundaries to be collected.

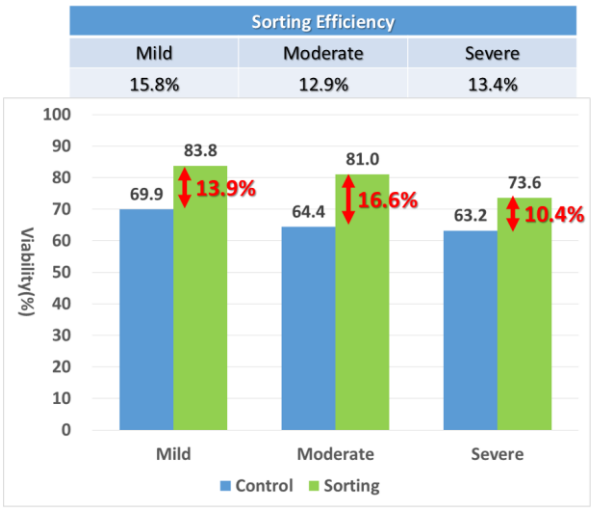

Fig.5 After sorting, the mild/moderate/severe oligozoospermia samples viability enhancements and sorting efficiencies. This chip is applicable for all kind of sperm concentrations.

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