BIOCHIP FOR SEPARATION OF MOTILE SPERM BY USING THERMOTAXIS

Yongjun Ko[1], Joon-Ho Maeng[2], Seung-Mo Ha[1], Seung Yong Lee[2], Yoomin Ahn[1], and Seung Yong Hwang[3,4]

[1]Department of Mechanical Engineering, Hanyang University, KOREA
[2]Department of Biochemistry, Hanyang University, KOREA
[3]Division of Molecular and Life Science, Hanyang University, KOREA
[4] GenoCheck Co. Ltd, KOREA

ABSTRACT

In this study, we present the novel microchip to separate a motile sperm using a thermotaxis resulting from temperature gradient in microchannel, without flow by an external device. Our microchip was fabricated by a replica molding technique using a PDMS (polydimethylsiloxane) and glass substrate. The micro heater for generation of temperature gradient was fabricated with the ITO (Indium Tin oxide) film. In consequence, temperature gradient was linearly generated by the micro heater within the microchannel. Under the optimal temperature gradient, some motile sperm vigorously moved toward the outlet well.

KEYWORDS: ITO, Microchip, Motile sperm, PDMS, Thermotaxis

INTRODUCTION

The motile sperm separation is essential process of the intracytoplasmic sperm injection (ICSI) which is most commonly used to overcome male infertility problems. The conventional sperm-sorting techniques are so performed by a hand without objective criterion that they are labor-intensive and error-prone manipulation. Currently, some types of microchip for overcoming these drawbacks have been reported. Kricka *et al*. compared sperm motility determined using disposable glass microchips and a conventional Makler chamber [1], Cho *et al*. reported a self-contained integrated microfluidic system that can separate motile sperm [2], and Horsman *et al*. demonstrated separation of sperm from a biological mixture containing epithelial cells on a microfluidic device [3]. These microchips mostly used external equipment such as syringe pump to separate a motile sperm, which can generate a laminar flow or a turbulence flow and interrupt the movement of motile sperm. Such an obstacle may ultimately provide lower the accuracy and efficiency of motile sperm separation. In this study, we developed the novel microchip to separate a motile sperm using a thermotaxis.

EXPERIMENTAL

Because thermotaxis, the phenomenon in which a cell directs its movement according to temperature gradient, is essential principle in our microchip, it is very important to generate a temperature gradient in the microchannel. As shown Fig. 1(a) and (b), the sperm separation chip was fabricated by a replica molding technique using a PDMS (polydimethylsiloxane) and glass substrate. The micro heater for gener-

978-0-9798064-2-1/μTAS2009/$20

ation of temperature gradient was fabricated on the ITO (Indium Tin oxide) glass by conventional photolithography and wet etching process.

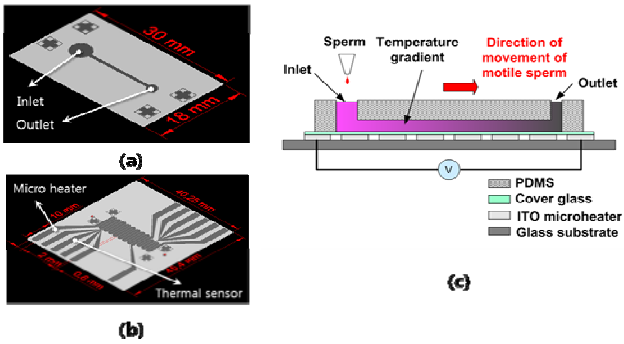


Figure 1. Schematic view of (a) sperm separation chip, (b) micro heater and (c) sperm separation experiment setup. In order to use the sperm separation chip as the disposable, the micro heater is designed to be separable from the chip and to be used repeatedly.

For sample preparation, epididymis was surgically extracted from the mouse, and then sperm cells were softly squeezed. The number of sperm cells was calculated by a hemocytometer. After that, they were stored in the tube with Human tubal fluid (HTF) medium at 37ºC. Fig. 1(c) shows a schematic view for separation of motile sperm. At first, after introducing pre-warmed HTF medium through an inlet, temperature gradient in the microchannel was generated by the micro heater for 5 min. 1 μL sperm solution was carefully dropped into the inlet, and then we maintained the setting condition for 13 min. Finally, when some motile sperms reached to the outlet by thermotaxis, the number of motile sperm was counted in outlet by a microscopy.

RESULTS AND DISCUSSION

To measure a temperature of inlet and outlet, thermal sensor was integrated in the middle of the micro heater. To evaluate heat distribution by the micro heater, heat transfer was simulated by commercial simulation program (CFD-ACE+, ESI Group). Fig. 2 shows that the linear temperature gradient was generated in microchannel through the micro heater.

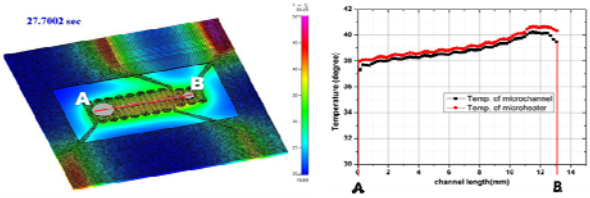
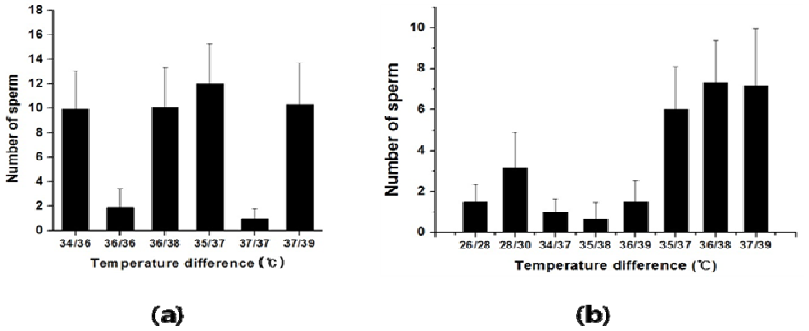


Figure 2. View of (a) simulated thermal profile and (b) temperature distribution of the sperm separation chip at 27.7seconds after applying voltage to the heater.

As shown Fig. 3(a), when temperature gradient was normally generated in microchannel, some motile sperm moved toward outlet. The result indicates that thermotaxis have an effect on sperm movement. Fig. 3(b) shows that temperature below 30ºC is negatively condition, and thermal gradient at temperature difference of 3℃ was more effective than that of 2℃.



(a) (b)

Figure 3. The number of sperm according to various temperature gradient conditions. (a) We could observe that thermotaxis have an effect on sperm movement, compared with controls (36 ℃/36 ℃ and 37 ℃/37 ℃). (b) Optimal condition for sperm mobility is thermal gradient at temperature difference of 2 ℃.

CONCLUSIONS

Consequently, temperature gradient was successsfully regulated by a micro heater and sperm separation chip were fabricated. Under optimal condition, temperature difference of 2℃, motile sperm was successfully separated by thermotaxis. Therefore, the proposed biochip can be powerful tool for separation of motile sperm in ICSI.

ACKNOWLEDGEMENTS

This work was supported by the Korea Research Foundation Grant funded by the Korea Government (MOEHRD, Basic Research Promotion Fund) (KRF-2006-311-D00326).

REFERENCES

[1] L.J. Kricka, I. Faro, S. Heyner, W.T. Garside, G. Fitzpatrick, G. McKinnon, J. Ho, and P. Wilding, J. Pharm, Micromachined analytical devices: microchip for semen testing, Biomed. Anal., 15, pp. 1443-1447, (1997).

[2] B.S. Cho, T.G. Schuster, X. Zhu, D. Chang, G.D. Smith, and S. Takayama, Passively driven integrated microfludic system for separation of motile sperm, Anal. Chem., 75, pp. 1671-1675, (2003).

[3] K.M. Horsman, S.L.R. Barker, J.P. Ferrance, K.A. Forrest, K.A. Koen, and J.P. Landers, Separation of sperm and epithelial cells in a microfabricated device: potential application to forensic analysis of sexual assault evidence, Anal. Chem., 77, pp. 742-749, (2005).