ANALYSIS OF SPERM QUALITY IN A MICROFLUIDIC DEVICE
Yu-An Chen,1# Ji-De Huang,1# Cheng-Ming Lin,1# Chang-Yu Chen,1# Vincent F.-S. Tsai,2# Chih-Kung Lee,1 Wen-Jong Wu,3 Ju-Ton Hsieh,2 Hong-Chiang Chang,2 Wei-Yuan Ma,1 and Andrew M. Wo,1#
1Institute of Applied Mechanics, National Taiwan University, TAIWAN
2Department of Urology, National Taiwan University Hospital, TAIWAN and
3Department of Engineering Science and Ocean Engineering, National Taiwan University, TAIWAN
# Authors contributed to this paper equally.

ABSTRACT
About 45% in infertile couples has been proven owing to male factor but the need of a rapid, easy to use, and precise sperm quality exam for men to self test at home has not been fully met.[1] This paper presents a microfluidics based sperm quality analysis device, which perhaps fits the requirement of an affordable and handy tool for the public to assay sperm quality. The proposed microfluidic device differs from commercial products in presenting sperm quality through an objective quantitative index instead of subjective qualitative readout. The proposed device may help infertile patients to trace their variation in sperm quality in a therapeutic process and couples planning to have a baby.

KEYWORDS: Sperm, Fertility, Resistive pulse technique

INTRODUCTION
The deteriorating trend of human sperm quality and high prevalence (about 10–20%) of infertility have been serious issues on human reproduction.[1-3] Diagnosis for a man suffering problem of infertility clinically focuses on examination of sperm quality, which is characterized by eight indices by the World Health Organization.[4] Among these indices, sperm concentration and motility could be the most critical two indices relating to fertility and usually first being checked. Attending outpatient clinics is currently necessary for most male suffering problem of infertility for examining these sperm quality indices. However, examination of sperm quality requires fresh samples provided by patients and thus sample acquisition in hospitals is unavoidable to patients. Much anxiety, embarrassment, and inconvenience may cause patients uncomfortable and even refuse subsequent visit if necessary. Moreover, since sperm quality of an individual man varies over time drastically perhaps due to stress, diet, and so on, infertility should be identified by tracing sperm quality over a period rather than only single exam.[4] For those identified as infertile patients, routinely recording time-varying sperm quality would benefit doctors to determine efficacy of therapy. Per discussion above, a need of convenient, informative, low cost sperm quality test for male self-exam sperm quality in comfort of home has been proposed but currently not been satisfied.

Commercial products of home use sperm examination rely on sperm staining and checking numbers of total or motile sperm concentrations. The result interpretations are similar with that of pregnancy midstream test so that only over or below a given threshold can be known. However, sperm quality is not a binary index. Hence, readout of the tests by judging colors might not be convenient to quantify sperm quality and thus not easy to long-term trace quality variation. Furthermore, ambiguous interpretation on the results might also be an issue.

This paper aims to provide a home use sperm quality test kit for the public to assay sperm quality at home. The proposed examination mechanism quantifies sperm quality in terms of the two critical fertility-related indices—sperm concentration and motility. Fertilization highly relies on large amounts of sperms with high motility. The quantified sperm quality index via the reported method is proportional to motile sperm concentration, which is particularly important to fertility. Prototypes of sperm quality chip and its reader are presented.

SPERM QUALITY CHECK BASED ON MOTILE SPERM CONCENTRATION
Figure 1a shows a photograph of microfluidic sperm quality chip. In three reservoirs A, B, and C, buffer solution and semen are held at distinct heights to drive flow field inside microchannel. Schematics in Fig. 1b indicates flow originating from channel A and then dividing into two streams: one flows along channel B and the other flows through an aperture then continues in channel C. At t=0, sperms initially placed at one terminal of channel B swim against flow and then being flushed through the aperture as shown in Fig. 1c. Two electrodes at terminals of channel A and C inject a steady current through the aperture and voltage drop between these two electrodes is continuously recorded for a period. Figures 1d and 1e show difference in pulse numbers induced by sperms would indicate sperm quality quantitatively. Pulse number represents sperm quality in terms of two critical fertility-related indices—sperm concentration and motility.

978-0-9798064-3-8/µTAS 2010/$20©2010 CBMS
14th International Conference on Miniaturized Systems for Chemistry and Life Sciences
3 - 7 October 2010, Groningen, The Netherlands
MATERIAL AND METHOD

Sperm samples were acquired from the National Taiwan University Hospital with the approval of the institute of review board. Semen was placed over 30 min for liquefaction after retrieval from the patients. Measurement was done before 6hr from sample acquirement to ensure viability of sperms.

Buffer solution was prepared by mixing RPMI 1640 with seminal plasma in a ratio of 4:1. Twenty percents addition of seminal plasma into RPMI1640 gains advantage of preventing sperms from adhering to glass surface. One micron diameter particles was inserted into buffer solution for visualizing flow field inside microchannels.

Photolithography was leveraged to fabricate master of microchannel for rapid molding. Photoresist AZ-P4620 was spun over a glass substrate followed by curing, exposure to UV light to transfer pattern of microchannel network from Cr mask. The developer for dissolving the exposed photoresist was mixed by AZ400K with water in a ratio of 1:4. Subsequent to development, dried master was poured over mixture of PDMS reagents A and B and placed for bubble removal. Cured PDMS block was cut off from the master and three holes for fluidic interconnection were punched at three ends of microchannel network. After plasma treatment, glass substrate was covered on PDMS block with microchannel in relief forming irreversible sealing. At top of the PDMS block, a PMMA block drilled with three holes was attached serving as reservoirs to hold semen and buffer solution at different heights.

Figure 2a presents a stand-alone prototype containing a disposable sperm quality chip, a reader, and a liquid crystal display module (LCM). Sperm quality quantified by pulse number will be shown on LCM. Figure 2b shows another type of the reader which is powered by PC and diagnostic results can be transferred and saved in PC through a USB cable.

Figure 3 shows architecture of the kit. Two electrodes on chip connect to a steady current source and voltage drop between these electrodes is digitized and processed by ICs programmed for pulse counting. Number of pulses induced within 12min after semen loaded on the chip is shown on LCM or user interface on PC.
Figure 4a shows a time course voltage presenting few pulses from beginning to about 200s followed by pulses induced frequently. The former period was spent by sperms to travel through channel B and pulses shown later were produced by sperms passing through the aperture. Figure 4b shows an enlarged portion of Fig. 4a from 600s to 680s. Each pulse represents one sperm being flushed through the aperture.

The proposed home use sperm quality analysis kit could be helpful to male with infertile problem. Simple, rapid, and economic sperm quality check by the proposed kit could be envisioned.

CONCLUSION

A microfluidic sperm quality analysis chip is proposed and two types of the reader are also presented. The flow field in microchannel tests swimming ability of sperms and those sperms traveling through 5mm long channel in a given time are counted via resistive pulse technique. The count of sperms enumerated during this time limit represents sperm quality in terms of concentration and motility. The reader was demonstrated and shows that wallet-size sperm quality test kit would be envisioned.

ACKNOWLEDGEMENTS

This paper is supported by the National Science Council (Grants NSC94-2212-E-002-054 and NSC98-2120-M-002-009).

REFERENCES


CONTACT

*Andrew M. Wo, tel: +886-2-33665656; Andrew@iam.ntu.edu.tw