SPERM QUALITY ASSESSMENT VIA SEPARATION AND SEDIMENTATION IN A MICROFLUIDIC DEVICE

Tsun-Chao Chiang[1], Chang-Yu Chen[1], Hao-kai Liu[1], Shu-Sheng Lin[1], Cheng-Ming Lin[1], De-Shien Jong[3], Vincent F.-S. Tsai[2], Ju-Ton Hsieh[2], and Andrew M. Wo[1]

[1] Institute of Applied Mechanics, National Taiwan University, Taipei, TAIWAN

[2] Department of Urology, National Taiwan University Hospital, Taipei, TAIWAN

[3] Department of Animal Science and Technology, National Taiwan University, Taipei, TAIWAN

ABSTRACT

About 45% couples with infertility have been inferred due to a male factor. Total concentration and motile percentage of spermatozoa are two major factors in semen quality assessment. Recently, many microfluidic devices have been developed to quantify these two factors[1, 2], but to the best of authors' knowledge, none of a device can evaluate both total and motile sperm concentrations in a single test. This paper presents a method of separation and sedimentation to assess both factors simultaneously in a microfluidic device. Results reveal good correlation with standard microscopic investigations.

KEYWORDS

Sperm analysis, Fertility, Microfluidic device, Cell counting

INTRODUCTION

The deteriorating trends of human semen quality and infertility have been serious issues on human reproduction in recent decade. About one out of six couples will visit the fertility department of the hospital since they have problems with getting pregnant. Male infertility is the main factor in ~46% of the case, while combination of abnormalities for both the man and woman account for the same percentage. 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. Among these factors, sperm concentration and sperm motility could be the most important factors relating to fertility and usually the first checked.

Before treatment of infertility, semen quality information is needed. Semen assessment is a first step in the investigation of the male fertility and given a description of the semen and contents. Currently, laboratory and hospital technicians still observe the performance of spermatozoon in semen through microscope with hemocytomer or Makler chamber, as a first step treatment of infertility couple. Thence several research groups have attempted to development novel technology or devices for semen assessment. So far, computer-aided sperm analysis (CASA) is developed with high level optical equipment and software which assess spermatozoon counts by size filter, motility by tracking program and morphology by stain. Beside, sperm quality analyzer (SQA) is visible in hospital and small laboratory. The SQA is for a quantitative evaluation of semen parameters using electrical optics to determine concentration and motile percentage. The assessments are not accurate and stable as CASA in low concentration and motility.

The paper presents a microfluidic device which judges two main factors for infertility treatment. The first issue is to develop a specific fluid pattern guided by microstructures. Second, the key concept of separating motile sperm in two different regions based on swimming feature of sperms. Third, the pellet area was from by sedimentation; the total concentration and motile percentage can be evaluated by pellet area analysis. Finally, we demonstrated the efficacy of the device, characterizing its ability to assess both total sperm concentration and motile sperm percentage simultaneously. This microfluidic device is suitable for sperm quality analysis without the aid of expensive microscope.

Design concept

Figure 1a illustrates the key concept of the microdevice composing of two channels separated by a phaseguide structure. Semen is loaded to the left channel in contact with buffer at right channel without disturbance. Presumably, all non-motile sperms will stay at left channel and motile sperm can swim randomly at both channels to achieve a dynamic equilibrium, i.e., 50% motile sperms stay in right channel. After centrifugation, pellet areas can describe sperm quantity[3], and both total and motile sperm concentration can be estimated based on calculation of occupied area. Figure 1b shows the device photo and enlarged regions of loading site and sedimentation sperm counter. Phaseguide structures[4] facilitate volume quantification and control the liquid filling sequence. Counter region collects sperms to form pellets after sedimentation. The semen and buffer loading sequence is presented in Fig. 1c. Loaded semen and buffer generate liquid-air meniscus along with phaseguide, and then buffer overflows the gap and interface contacts gently. Figure 1d presents the entire experimental protocol. Semen and buffers are loaded in chip and wait for dynamic equilibrium. Chip is then fitted in tube for centrifugation, and images of resulted pellets are captured and analyzed.

Dynamic equilibrium of motile sperms

The time required to reach dynamic equilibrium of motile sperms was experimentally verified in a wide range of motile sperm concentrations. Video recording of the buffer region of the main channel would reveal the equilibrium

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

process. Figure 2 shows the number of sperms appeared in the recording area analyzed every minute for 20mins. No sperm was observed initially, and number of recognized sperms reached an equilibrium value gradually. Regardless of motile sperm concentration among five samples, dynamic equilibrium of motile sperms was achieved after about 14 minutes.

The dynamic equilibrium process of motile sperms can be modeled analogous to particle diffusion. Diffusion coefficient (Ds) of motile sperms was roughly estimated by $D_s = \frac{\text{constant step length}}{\text{time for each step}} = \frac{\ell^2}{\tau} = \frac{65\mu m^2}{1s} = 4.225 \times 10^{-12}\ m^2/s$. An analytical solution for diffusion in one-dimension was applied to calculate the mixing scenario, i.e., motile sperms evenly distribution in the entire main channel. The entire width of the main channel was 3 mm, while sperms occupied only 1.2 mm initially. Computation showed the percentage mixing of motile sperm reached 98% after 14 mins, which agreed well with experimental observation. Hence, 15 mins of equilibrium time was applied for all experiments in this work.

Optimization of sedimentation procedure

Relative centrifugal force (RCF) is one of the important factors to form compact and robust pellets. Figure 3 shows evolution of sperm pellet area with increased RCF from four samples with different concentrations. Inserted photos indicate the process during the pellet development. Over 1000xg is considered sufficient centrifugal force for a constant pellet formation. The error bars represent standard deviation from three independent tests all with 5 mins centrifugation.

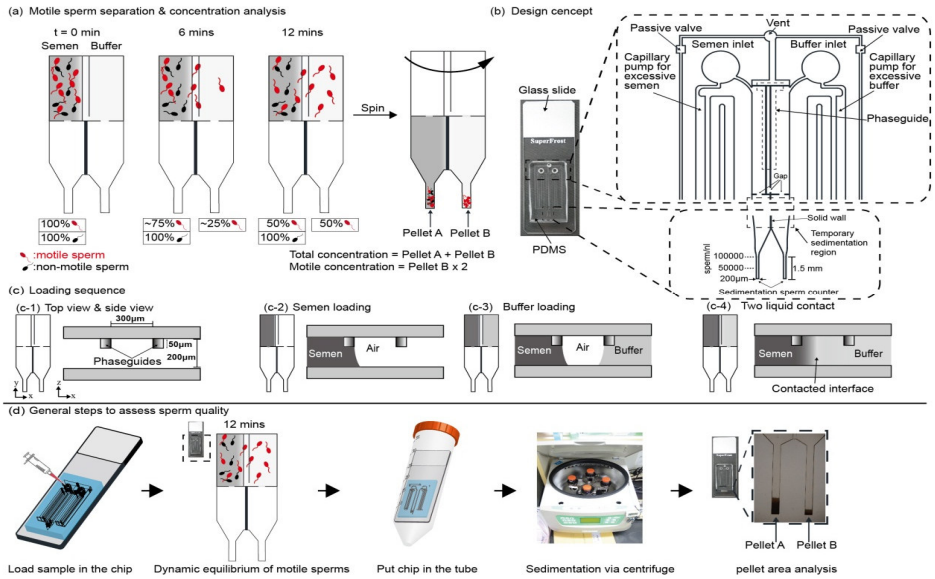

Figure1. Design concept and experimental process. (a) Method of calculating both total and motile sperm concentration (b) Design of the microfluidic sedimentation counter. (c) Illustration of liquid filling sequence. (d) General steps for sperm quality assessment.

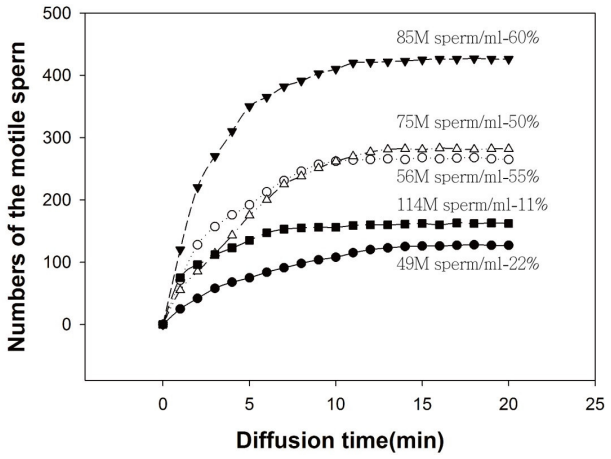

Figure 2 Diffusion time of motile sperms

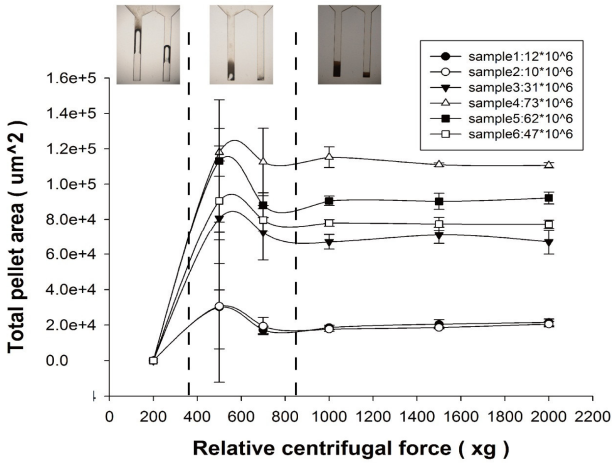

Figure 3 Pellet evolution under different relative centrifugal force.

Assessment of sperm quality

Total concentration of sperms is the first factor that needs to be addressed for its quality assessment. After characterizing sample loading, dynamic equilibrium, and sperm sedimentation, numbers of samples with different concentration and motile percentage were tested. Total pellet area (Pellet A+ Pellet B) in the SSC, which represented total sperm concentration, was compared to the concentration obtained from manual counting on a Makler chamber, as shown in Figure 4. The error bars represent standard deviation from three independent repeats of the same sample. The linear regression revealed good correlation with coefficient of determination R^2= 0.97. In other words, total pellet length was directly proportional to the sperm concentration in the testing range of 15-110 million sperms per milliliter. Besides, results also specified that every 1 μm pellet increment indicates ~290 sperms stack in the SSC. Since the total sampling volume was 2.3 μl, this implied the resolution of sperm counting was $290 \times 2 \times \frac{1000}{2.3} = 0.25$ M sperms/ml under ±1 μm image resolution in this study.

Motile sperm percentage was evaluated by calculating the relationship of the two SSC channel [Pellet B×2/(Pellet A+Pellet B)]. Figure 5 compares the motile percentage from pellet analysis and Makler chamber. The assessment evaluated motile percentage in range of 10-70 % which covers the cutoff value in 40% by WHO. A good coefficient of determination showed R^2= 0.84 with respect to the theoretical relation y=x. In addition, most cases revealed standard deviation of within ±5%. The two deviated cases around 20-30% motile percentage showed slightly higher motile percentage. It was conceivably caused by the minor disturbance during the sedimentation process due to imperfect chip fabrication.

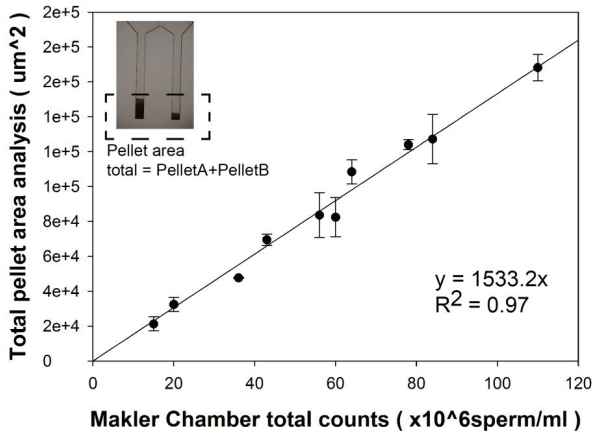

Figure 4. Sperm total pellet area compared to total sperm counts from Makler Chamber.

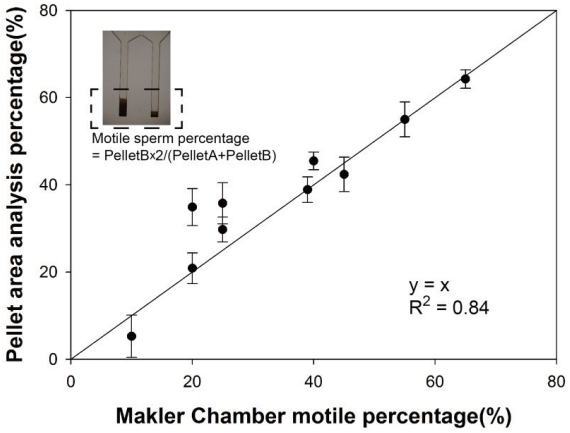

Figure 5. Sperm motility from pellet analysis compared to motile percentage from Makler Chamber.

Conclusion

We demonstrated the design, fabrication, and characterization of microfluidic device for assessing spermatozoa quality. The designed phaseguide structure has been employed in this work to generate desire fluid patterns for sample loading. In addition, the device utilized separated and sedimentation concept to evaluate the sperm quality. Experimental protocol for stable and accurate results was also optimized. Unlike other sperm-counting techniques, the device requires no high level microscope and training of technician. It is conceivably an accurate and easy approach for sperm quality assessment.

REFERENCES

[1] Y. A. Chen, Z. W. Huang, F. S. Tsai, C. Y. Chen, C. M. Lin and A. M. Wo, Microfluid Nanofluid, 2011, 10, 59-67.
[2] L. I. Segerink, A. J. Sprenkels, P. M. ter Braak, I. Vermes and A. van den Berg, Lab Chip, 2010, 10, 1018-1024.
[3] J. L. Garcia-Cordero, L. M. Barrett, R. O'Kennedy and A. J. Ricco, Biomed Microdevices, 2010, 12, 1051-1059.
[4] P. Vulto, S. Podszun, P. Meyer, C. Hermann, A. Manz and G. A. Urban, Lab Chip, 2011, 11, 1596-1602

CONTACT

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