ShuQi Wang, Fatih Inci and Utkan Demirci | January 14, 2015 | 1 Comment

INFERTILITY IS A SIGNIFICANT CLINICAL ISSUE

It is reported that 5.3 million American couples of reproductive age are affected by infertility. Among these couples, male factors account for up to 50%, necessitating the identification of key parameters dictating male fertility, including sperm count, morphology and motility. Assisted reproductive technologies (ART) have emerged as powerful tools to address male infertility problems in modern clinical practice. Up to now, *in vitro* fertilization (IVF) with or without intra cytoplasmic sperm injection (ICSI) has become the most widely used assisted reproductive technology in modern clinical practice to overcome male infertility challenges. One of the obstacles of IVF and ICSI lies in identifying and isolating the most motile, and the healthiest sperm from semen samples that have low sperm counts (oligozoospermia), low sperm motility (oligospermaesthenia) and/or abnormal sperm morphology (teratozoospermia). Selection of the best performing sperm based on the selection criteria including motility and morphology is the keystone for successful outcomes of fertilization and full term pregnancy. However, it remains a clinical challenge to select the most motile normal/healthy sperm from oligozoospermic, teratozoospermic or oligospermaesthenic samples. Traditional sperm selection techniques, such as the swim-up method and density gradient-based centrifugation, have been widely used to separate highly motile sperm from the rest of the sample [1, 2]. However, neither of these techniques is optimal for healthy sperm selection. Briefly, the swim-up method does not provide a high yield of motile spermatozoa, and the centrifugation procedure may have a deleterious effect on sperm. Although the density gradient separation approach can be selective, it has been shown to produce high sperm DNA fragmentation and ROS generation [3, 4]. Clearly, a novel approach to efficiently isolate the most motile and healthy sperm is urgently needed, thus increasing ART success rates.

With rapid advances in microfluidics technologies, miniaturized devices have been widely used to address a variety of clinical needs, since they offer precise control of fluid dynamics. Nevertheless, there are no microfluidic technologies that can simultaneously track, identify and sort the most motile sperm for subsequent IVF. Two major challenges have been well recognized. First, a small field of view (FOV) in conventional microscopes significantly limits the capability to track ~0.5 million motile sperm in 5-20 microliters of sample when they are in active motion. Second, there is a lack of simple and cost-effective microfluidic sorting devices that can be performed by healthcare workers in a clinical setting to facilitate IVF. When using currently available microfluidic devices for sorting healthy sperm, chemical stimuli and controlled flow using peripheral microfluidic pumps are needed.

BIOMEDICAL APPROACHES FOR IMPROVING ASSISTED PREGNANCY

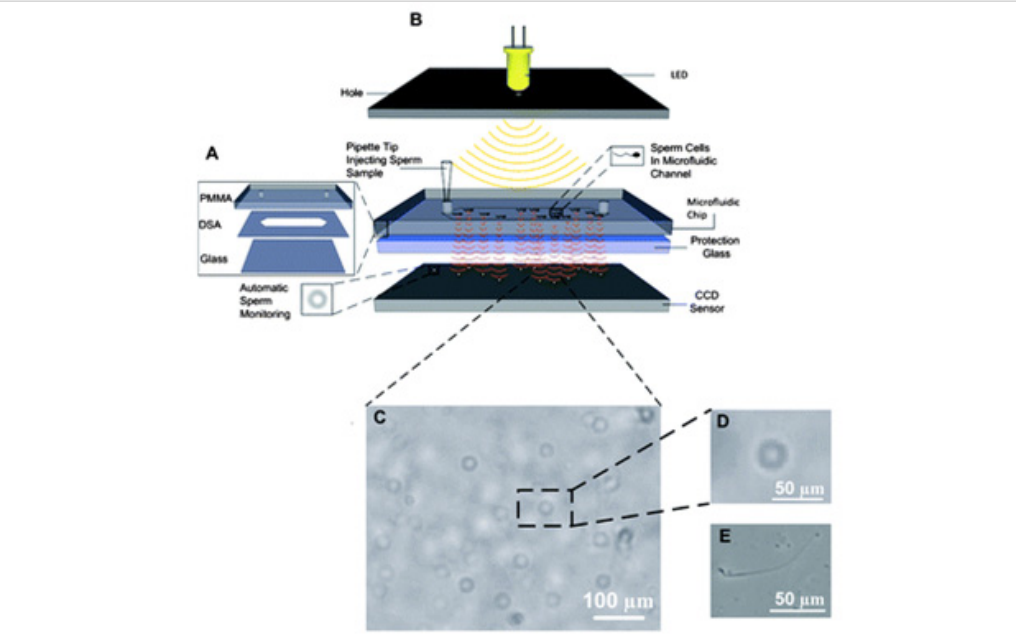

Figure 1. *Schematic of a lensless CCD system for imaging sperm in microchannels.* ***(A)*** *Assembly of microchips using PMMA, DSA, and glass coverslip.* ***(B)*** *The lensless CCD system coupled with microchips for sperm tracking.* ***(C)*** *Shadow image of sperm in a microchip obtained using the lensless CCD system. Scale bar is 100 mm. (D) Magnified shadow image of a spermatozoa* ***(C)****. Scale bar is 50 mm.* ***(E)*** *Microscopic image of sperm at 10x objective. Scale bar is 50 mm (Reproduced from Ref. Zhang et al. 2011with permission from The Royal Society of Chemistry).*

To address the first challenge, we developed a lensless imaging-based tracking system [5]. This system consists of disposable microchips and a lensless charge-coupled device (CCD). The microchip has a dimension of 24 mm x 40 mm and is fabricated using a non-lithographic technology. The microchip consists of three layers from top to bottom: Poly(methyl methacrylate) (PMMA), double-sided adhesive (DSA), and glass coverslip (**Figure 1A**). In the microchip, a microchannel with an inlet and outlet was imbedded. The microchip was positioned on a lensless CCD with light shed above to obtain shadow images of sperm (**Figure 1B**). A series of shadow images were obtained using the lensless CCD at a rate of one frame per second (**Figure 1C**). Compared to microscopic imaging, the lensless CCD has a wide field-of-view to image all the sperm throughout the entire channel. The motility of sperm was recorded in real-time. Similarly, the entire system can be imaged vertically by rotating the system 90 degrees, which is a great advantage compared to traditional microscopic imaging. Thus, we can reliably assess the motion of sperm in both configurations.

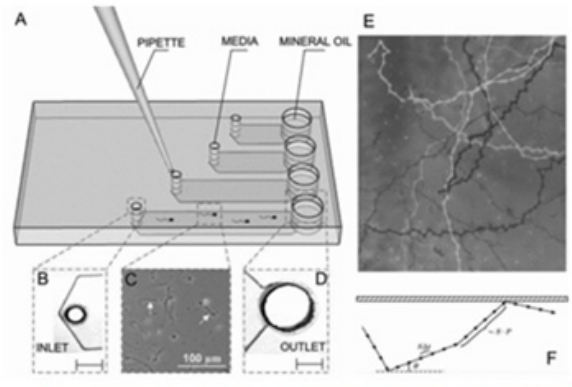

Figure 2. *A schematic of space-constrained microfluidic sorting (SCMS) system.* ***(A)*** *The SCMS microchip has different channel lengths for effective sperm sorting.* ***(B)*** *A microscope image of the inlet under a 2X objective.* ***(C)*** *A microscope image of a microchannel under a 10X objective.* ***(D)*** *A microscope image of the outlet under a 2X objective. Scale bars for the channel inlets and outlets are 1 cm.* ***(E)*** *Sperm tracking with the aid of ImageJ (NIH).* ***(F)*** *A schematic of the trajectory of a sperm (Reproduced from Ref.* ***Tasoglu et al.*** *2013 with permission).* ***(G)*** *FERTILE-ICSI product.*

Further, we addressed the second challenge by developing a space-constrained microfluidic sorting (SCMS) system for selection of the most motile sperm [6]. In this flow- and chemical-free, naturally-inspired biodesign (**Figure 2**), we evaluated different channel lengths and incubation periods for maximizing the sorting capability for the most motile sperm. By designing the SCMS system with respect to channel length and incubation time, we can sort out the most motile sperm with high efficiency. We found that the most motile human sperm could be collected using such a chip with a channel length of 20 mm for 1 h incubation. For convenient sperm collection, a larger outlet (diameter of 2 mm) can be designed. After sorting, the most motile sperm reaching the outlet can be manually pipetted out from the outlets and counted. Such a simple and efficient sperm sorting system can be easily implemented at clinics to facilitate IVF.

In addition, we developed a macro-microfluidic sperm sorter (MSS) that mimics the natural vaginal sperm sorting mechanisms, where sperm migrate towards the egg through “microchannels” formed by vaginal mucus [7]. Thus, the most motile and healthy sperm can naturally reach the egg and complete fertilization. By mimicking this process, we designed a porous filter sperm sorting microchip in which a polycarbonate nucleopore track-etched membrane was imbedded (**Figure 3**). For optimal sperm sorting, we assessed different pore sizes (3, 5 and 8 μm). After injecting 560 μL of unprocessed semen, the chip was incubated at 37ºC for 30 min before the sperm was collected from the outlet after filtration. We observed that the sorted sperm had significantly less ROS and DNA fragmentation than those sorted by the conventional swim-up method [7]. Thus, the most motile and functional sperm were selectively sorted through this filtration process using a disposable microchip, while the dead or less motile sperm were retained by the filter membrane. The presented microchip is easy-to-use, highly efficient, and can standardize sperm sorting without centrifugation in clinical settings; thus, eliminating sorting variations between operators.

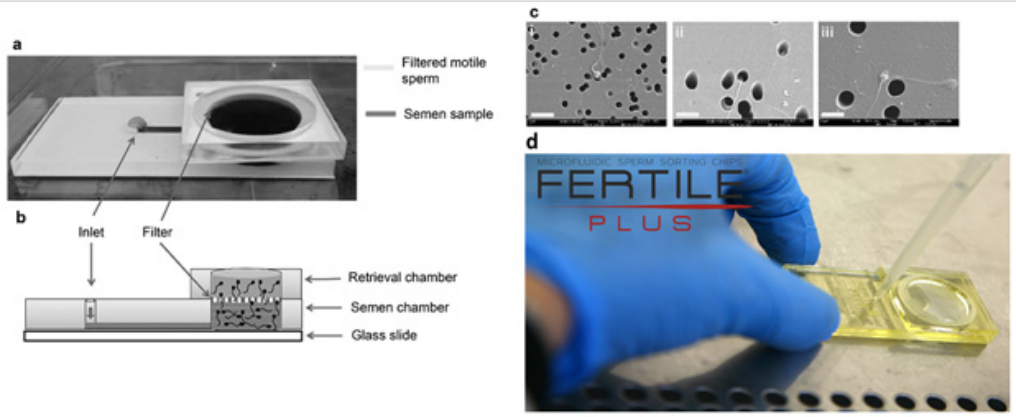

Figure 3. *Selection of functional and motile human sperm on-chip. a) Micrograph of sperm sorting microchip. b) Principle of sperm sorting microchips. Once injected into the inlet, the most motile and healthy sperm can swim the filter membrane with a pore size of 3, 5 or 8 μm. c) SEM images of sperm passed through polycarbonate nuclepore track-etched membrane i) 3 μm, ii) 5 μm, and iii) 8 μm. The scale bar is 10 μm (Reproduced from Ref. Asghar et al. 2014 with permission). d) FERTILE PLUS-IUI product.*

Our simple and cost-effective microfluidic design for sperm sorting, in which motile morphologically healthy sperm can be effectively separated by a space-constrained microfluidic sorting (SCMS) chip is significantly needed at the clinic. This method offers a flow-free and chemical-free system in patient care for sperm sorting and would increase the success rate of ICSI/IVF for male factor, which in turn would yield a substantial improvement of ICSI/IVF outcomes. Given that clinical reproductive medicine has been a challenging and labor-intensive field, adoption of such an easy-to-use micro-device will significantly lead to improvements in clinical outcomes and decreased dependence on operator skills, facilitating repeatable and reliable operational steps.

Today, ICSI and Intrauterine insemination (IUI) have become most commonly used for assisted pregnancy. Our Chip-ICSI and Chip-IUI can be used to select the most motile sperm for ICSI and IUI, respectively. This Chip-ICSI (*i.e.*, commercialized as FERTILE™) eliminates manual variations from operator to operator and standardizes the sperm selection process in the clinic. The Chip-IUI (*i.e.*, commercialized as FERTILE PLUS™) replaces conventional methodologies such as centrifuge-based processes, and hence, enables the selection process to be successfully performed in the office of an OB/GYN in a small town without enhanced facilities.

ART MICROCHIP TECHNOLOGIES

The FERTILE and FERTILE PLUS technologies are being commercialized by Koek Biotechnology and DxNow Inc., and tested in a few clinics in Turkey. Recently, Acibadem IVF clinic of Turkey reported up to a 50% increase in the pregnancy rates in the first clinical trials using the Chip-ICSI (FERTILE) relative to existing clinical methods. With these two advanced technologies, we will be able to assist millions of couples facing infertility issues.

In conclusion, the Chip-ICSI and Chip-IUI are expected to significantly advance the clinical practice of assisted reproduction. The translation of these microfluidic chips into commercially available medical devices will help millions of families worldwide avoid the stress of going through multiple IVF treatments.

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Competing Financial Interests

Dr. Utkan Demirci is a co-founder of, and has an equity interest in: (i) DxNow, Inc., a company that is developing microfluidic and imaging technologies for point-of-care diagnostic solutions, forensic applications, and assisted reproductive technologies; and (ii) Koek Biotech, a company that is developing assisted reproductive technologies for clinical solutions.

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