Application of microfluidic technologies to the quantification and manipulation of sperm

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1. Introduction

Taiwanese society has been facing a difficult situation with respect to low birth rate for many years. This low birth rate will affect the development of the country’s population and economy. This issue has attracted much attention in both the private and public sectors. As a result, the issue of increasing the birth rate has been emphasized as a concern in Taiwan’s national security. With respect to raising the birth rate, many aspects need to be considered. Although some couples use contraception for economic and social reasons, there are still many couples who cannot conceive as a result of infertility. In some studies, the prevalence of infertility has been reported to be >20%. Fifty percent of the causal factors in infertility can be attributed to male infertility. Helping these couples to overcome male infertility should be an urgent mission for urologists.

With respect to male fertility, sperm play a crucial role in the whole process of fertilization. Regardless of whether the pregnancy is spontaneous or assisted, the sperm count (defined as the total number of sperm in an ejaculate) and motility reflect the success rate of fertilization. Many technologies have been developed over the years to manipulate sperm, focusing on the counting and sorting of sperm. Microfluidic technologies were introduced in this field at the beginning of the 21st century. This paper reviews the history of microfluidic technologies and their application to forensic medicine and sperm counting and sorting. An attempt is made to establish an automatic platform for in vitro fertilization using microfluidic technologies.

2. History

The earliest review article about the application of microfluidic technologies to assisted reproduction technologies (ART) was published in 2002. Compared with other well-known ART technologies, microfluidic technologies are fairly new and are attracting a great deal of attention as a result of their potential applications in ART.

Throughout the current decade, microfluidic technologies have been implemented in a number of ART sectors, including the detection of sperm (such as in forensic examinations of cases of sexual assault), in sperm sorting, as a platform for fertilization and embryonic development (IVF-on-a-chip), germ cell manipulation, and as a platform for studying the processes and environment of fertilization. Some study groups focus entirely on microfluidic
technologies in ART — for example, Smith et al12 from the University of Michigan, Ann Arbor, MI, USA, van den Berg and coworkers13 of the University at Twente, Enschede, The Netherlands, and researchers from Peking Tsinghua University, Beijing, China.14 These study groups have published many papers demonstrating the potential of using microfluidic technologies in ART. As a result of the complexity of implementing a microfluidic platform, such study groups are always multidisciplinary and include experts in biomedicine and engineering. This collaboration between people of different backgrounds generally increases the success of novel systems.

Our group is also an example of such team work. Wo and coworkers15 develop a microfluidic platform for implementing sperm counting and sorting systems. Although there has been some use of microfluidic technology in sperm manipulation, improvements and new trials are still underway.

3. Applications in forensic medicine

It was shown in 2005 that microfluidic technology can be used to separate the epithelia of victims and the sperm of perpetrators in sexual crimes.18 Microfluidic methods are less time consuming and require fewer personnel than traditional separation methods. In 2006 Bienvenue et al19 set up a platform for sperm cytolysis and DNA extraction. Such a platform, incorporating the relevant specimen, reactive reagents, and a fabricated microfluidic configuration, can provide a complete analytical process as a series of traditional bench tasks. This process has been called “lab-on-a-chip” and is primarily based on microfluidic technologies.20

4. Sperm counting

In the early stage of developing a microfluidic sperm counting device, fluorescence was used to identify sperm cells for quantification.21 However, fluorescence and other staining methods are too complex to be used in home sperm tests. Therefore some technologies that do not use staining are emerging. Segerink et al13 developed a platform of microfluidic and electrical impedance technologies for sperm counting without staining. This platform differentiates differently sized cells, including sperm cells, by measuring the change in electrical impedance when the cells pass through a specific gate. In the same year, we developed a microfluidic system (Fig. 1) that not only utilized electrical impedance technology, but also the phenomenon of oriented sperm swimming (OSS) to count sperm.15 The phenomenon of OSS was first described over 100 years ago.22 However, the possible mechanism of OSS has not been clear until now. After observing the pattern of movement of sperm in microfluidic devices, a hypothesis to explain the mechanism of OSS has been proposed as follows. The head and tail of a sperm have different characteristics. The head is oval-shaped and contains organelles and the nucleus, which are higher density materials. Conversely, the tail is a long filament and contains lighter materials. This different distribution in density between the head and tail causes the passive orientation of the sperm in a stable stream (the head towards the upstream direction and the tail towards the downstream direction; Fig. 2). This phenomenon is similar to that shown with the use of a wind gauge.

A similar technology called the resistive pulse technique has also been demonstrated.16 Using this technique, some important characteristics of sperm motility can be extracted (i.e., the swim velocity and the tail-beat frequency). As a result of the simplicity of this technique, which require only a current/voltage source and data analysis, the resulting device is a step in the continuing evolution away from optical or visual methods for counting sperm.25

Another configuration of microfluidic device for assessing the total number of sperm and the percentage of motile sperm is based on the principles of the sperm’s random swimming and sedimentation.17 This platform can also provide information about the numbers of motile and immotile sperm.

A commercial microfluidic cell counter is currently available.26 This cell counter is designed to detect various kinds of cell. The range of cell size detected is 3–25 μm. However, with robust

![Fig. 1. Microfluidic chip for sperm counting.](image1)

![Fig. 2. Sperm swim before and after orientation (upper and lower panels, respectively) as a result of the different distributions of density between the head and the tail. Arrows on the left-hand side show the flow direction and the solid arrow indicates sperm swimming against the flow.](image2)
5. Sperm sorting

One of the purposes of sperm sorting is to enhance the number of good quality sperm to increase the rate of fertilization and pregnancy in ART processes such as IVF and intracytoplasmic sperm injection. Conventional sperm preparation techniques, such as swim-up, density gradient centrifugation, and glass wool filtration, are not efficient enough to produce the expected sperm populations for ART. There is therefore a need to develop efficient and effective methods of sperm sorting. The study team at the University of Michigan is one of the leaders in microfluidic sperm manipulation. They have demonstrated a pump-less hydrostatic pressure microfluidic device in which the sperm swim through laminar flows to reach a collecting area for motile sperm. This device differentiates motile sperm from immotile sperm, dead sperm or dead cells, and debris in raw semen, and thus opened a new era of microfluidic sperm sorting. This device differentiates motile sperm from immotile sperm, dead sperm or dead cells, and debris in raw semen, and thus opened a new era of microfluidic sperm sorting.

Subsequently, many microfluidic sperm sorting systems have been introduced. Shao et al. used laser trapping to quantify the motility of sperm and to select motile sperm on a microfluidic device. Zhang et al. established a platform integrating a lens-less charge-coupled device with a microfluidic chip for automatic recording as the sperm moved inside a microfluidic channel. Motile sperm were sorted by swimming through the outlet of a microfluidic channel.

In 2011 our team fabricated a 540 microchannel disk of glass, polydimethylsiloxane, and polymethylmethacrylate. This high throughput sperm sorting disk can sort motile sperm in 30 minutes, increasing the motile percentage from below 60% to 90%. In addition, the sorted sperm showed a better DNA integrity (tested by terminal deoxynucleotidyl transferase dUTP nick-end labeling) than sperm prior to sorting. This improvement in DNA integrity by sperm sorting was also described by Song et al. Matsura et al. later showed that a microfluidic sperm sorting system could reduce the treatment time for intracytoplasmic sperm injection.

6. IVF-on-a-chip

6.1. Modeling of the fertilization process

Clark et al. designed a microchannel to mimic the environment of fertilization (oviducts) that allows sperm to swim towards the ovum. This biomimetic microchannel reduced polyspermy and subsequently increased the number of potentially viable embryos without reducing the overall in vitro production efficiency. Suh et al. discovered that murine IVF could be conducted successfully within microfluidic channels with lower total numbers and concentrations of sperm.

With regard to the simulation of the fertilization process, Ko et al. from South Korea reported a microfluidic platform using acetylcholine as a chemoattractant to evaluate the chemotactic response of sperm.

As little is understood about the mechanism of guiding sperm to the ovum in the female reproductive tract, modeling the environment of fertilization is designed to explore all aspects of the fertilization process. Several mechanisms have been suggested, including chemotaxis, thermotaxis, and rheotaxis. Miki et al. using microfluidic techniques, found that rheotaxis is a major determinant of sperm guidance over long distances in the mammalian female reproductive tract. This is a good example of modeling the fertilization process using microfluidic techniques to explore the details of reproduction. Such approaches could be developed to implement an IVF process on a microfluidic chip. This technology is referred to as “IVF-on-a-chip”.

6.2. Robotic-assisted reproduction

Following the concept of IVF-on-a-chip, several research teams have proposed preliminary designs for robotic-assisted reproduction, which refers to the automatic processes of sperm sorting, fertilization, and embryo development. For example, a team from Peking Tsinghua University developed a single-oocyte positioning system integrated with motile selection and early embryo development on microfluidic platforms after the measurement of the motility of sperm and chemotaxis.

Meseguer et al. have summarized the present technologies available for implementing a robotic-assisted reproduction platform. These are: (1) sorting of sperm based on viability; (2) ovum denudation by the mechanical removal of cumulus cells with microfluidics; (3) precision ovum positioning by joystick-controlled micromanipulators; (4) microfluidics for the gradual change of a culture medium, resulting in better embryo development and the reduction of embryo manipulation; and (5) time-lapse, proteomic, and metabolic evaluation of the developing embryo to select the optimum embryo. With such technologies, a system of IVF-on-a-chip can be established that integrates sperm sorting and ovum positioning, thus facilitating fertilization and early embryo development. This means that a sperm and ovum can be pre-loaded on to the chip and a perfect embryo for implantation can be expected.

7. Future challenges

7.1. Fabrication and commercialization of devices

Like many mature in vitro diagnostic techniques that are available commercially, microfluidic devices need robust evolution and development from the initial idea to a prototype, and then on to mass production. There are always inevitable gaps between bench work in the laboratory and the final fabricated product. These gaps may involve aspects of design, materials, and machinery. For example, there was a quasi-microfluidic device called Fertell available on the British market, which used larger gauge tunnels for sperm to swim up and cause a reactive color change on a nitrocellulose strip. However, the Fertell device is no longer on the market in any country because this device had a high failure rate at each of the 17 steps in assembly. High failure rates in fabrication made the overall cost of manufacturing the Fertell device very high and the resulting product (that tested both the number and motility of sperm) could not compete in the marketplace.

Another hurdle in the commercialization of microfluidic devices for sperm analysis and other IVF-on-a-chip technologies is the lack of a commonly available interface for these chips. This leads to high costs in developing accessory laboratory equipment (such as syringe injectors, power sources, and voltmeters) in addition to the key microchip. Therefore one of the measures required to facilitate commercialization is the development of platforms compatible with existing off-the-shelf instruments (the so-called Microfluidic Apps).

With respect to these hurdles, it is necessary for the developers of microfluidic devices to take design for subsequent fabrication and commercialization into consideration at the bench work stage.

7.2. Acceptance by doctors and patients

Despite the advancement in sperm counting based on microfluidic technologies, there is still a large discrepancy between the
daily clinical usage and bench work of these technologies. There has been no substantial substitution for the accepted gold standard of microscopic techniques and technologies, such as manual and computer-assisted methods, for sperm counting. One of the reasons for such a slow evolution in semen analysis is that newly developed methods need to be verified through well-designed trials (accuracy) and they should fulfill users’ requirements for privacy and convenience.

With respect to sperm sorting and IVF-on-a-chip, IVF laboratories often resist change to maintain consistency in their laboratory conditions. However, the implementation of new technology is often necessary to explore scientific discoveries and to improve on earlier successes to advance the field of ART. Microfluidic platforms represent a technology that has the potential to revolutionize the fundamental processes of IVF, including handling gametes and embryo culture. Therefore IVF laboratories and the relevant staff should be open-minded when encountering such a newly developed technology.

8. Conclusion

Microfluidic technologies are still a relatively new field, especially in ART. Some successful implementations for sperm counting and sperm sorting have been demonstrated. However, there are still some obstacles to fabricating and commercializing microfluidic devices and obtaining acceptance from the relevant staff and users. It is therefore important to be open-minded and to continue the development of this new technology.

Conflicts of interest

The authors declare that they have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in the manuscript.

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References