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Review article

Application of microfluidic technologies to the quantification and manipulation of sperm☆

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ABSTRACT

With respect to male fertility, sperm play a crucial role in the whole process of fertilization. Many technologies for manipulating sperm have been developed over the past few decades and these have focused on counting and sorting sperm. Microfludic technologies were introduced into 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.

1. Introduction

Taiwanese society has been facing a difficult situation with respect to low birth rate for many years.[1] 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.[2]

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%.[3,4] Fifty percent of the causal factors in infertility can be attributed to male infertility.[5] 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.[6–8] Many technologies have been developed over the years to manipulate sperm, focusing on the counting and sorting of sperm.[9,10] Microfludic technologies were introduced in this field at the beginning of the 21st century. We review here the history of microfluidic technologies and their application to forensic medicine and sperm counting and sorting. An attempt is also made to establish an automatic platform for *in vitro* fertilization (IVF) using microfluidic technologies.

2. History

The earliest review article about the application of microfluidic technologies to assisted reproduction technologies (ART) was published in 2002.[11] 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.[10]

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

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technologies in ART – for example, Smith et al[12] from the University of Michigan, Ann Arbor, MI, USA, van den Berg and coworkers[13] 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 coworkers[15–17] from the National Taiwan University, Taipei, Taiwan have developed 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 al[19] 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 al[13] 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.[22–24] 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]

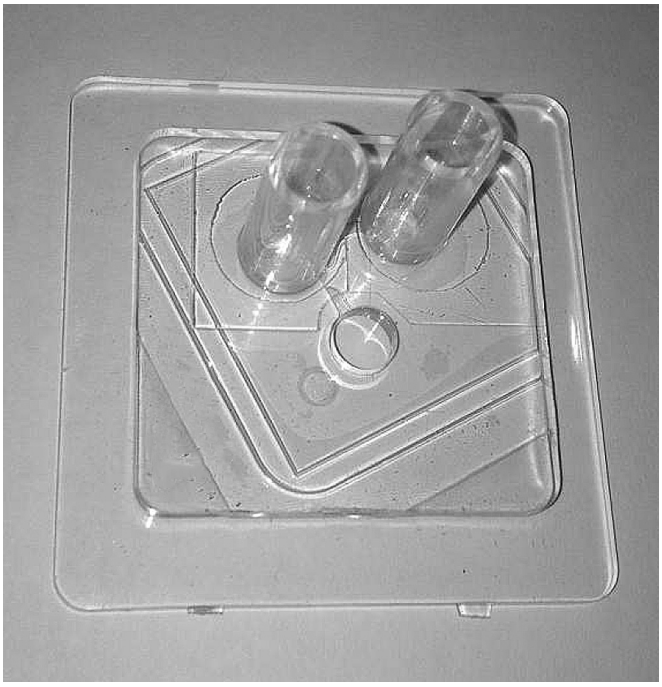

Fig. 1. Microfluidic chip for sperm counting.

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

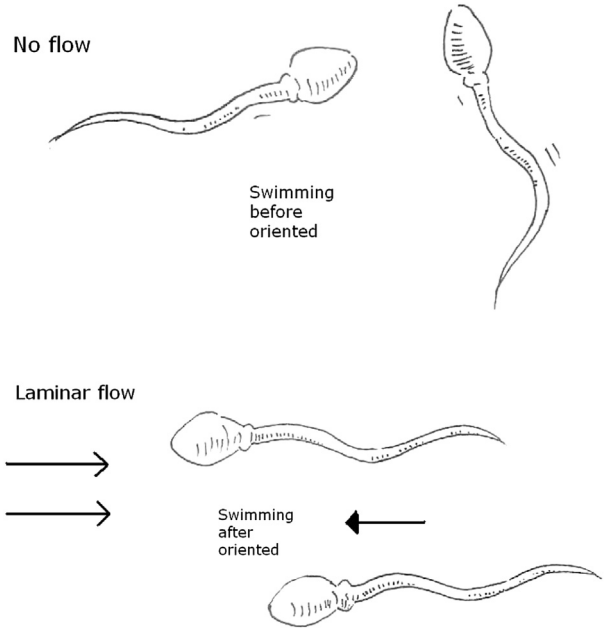

Fig. 2. Sperm swim before and after orientation (upper and lower panels, respectively) as a result of the different distributions of density between the head and the tail. Arrows on the left-hand side show the flow direction and the solid arrow indicates sperm swimming against the flow.