Sperm Selection

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Sperm selection is a clinical need for guided fertilization in men with low-quality semen. In this regard, microfluidics can provide an enabling platform for the precise manipulation and separation of high-quality sperm cells through applying various stimuli, including chemical agents, mechanical forces, and thermal gradients. In addition, microfluidic platforms can help to guide sperms and oocytes for controlled in vitro fertilization or sperm sorting using both passive and active methods.

Keywords: microfluidics ; lab-on-a-chip ; sperm sorting ; fertility

1. Introduction

Microfluidics and lab-on-a-chip devices play important roles in biology and medicine. Owing to their micron-sized features, such devices are not only capable of processing samples at low volumes (mL to nL) [1] but also allow for the possibility of sample manipulation in the microchannels. Microfluidic platforms enable various tests in a fast and low-cost fashion, using miniaturized or portable devices. This is of great importance for applications, such as single-cell analysis, drug encapsulation, drug and toxicity testing, separation and detection of biomarkers, and cell sorting [2][3][4][5][6]. The latter has attracted more attention recently due to the microfluidic systems' high precision and ease of performing steps, such as culturing, mixing, labeling, attachment to nano- and micro-particles, immune- or aptamer-based capturing, and separation of cells and stem cells. In addition, microfluidic systems can also provide platforms for studying the effects of chemical, physical, and mechanical stimuli on the cells, as well as advanced omics and metabolite analysis [5][7][8][9][10].

Infertility is a major healthcare problem, which affects 8–12% of couples worldwide. An important issue during conception is the selection of the best gametes. Scientists have been trying for years to enhance the chance of conception using various approaches [11][12]. Sperm, known as the male gamete and produced through gametogenesis in mammalians, plays a vital role in transferring the genetic materials of the father to the offspring. Following fertilization, the proteome of an oocyte cytoplasm is reprogrammed to start cell division and embryogenesis [13][14][15]. The generation of the mammalian gametes, which are derived from a founder population of primordial germ cells (PGCs), is determined early during the embryogenesis before they start their unique development process [16][17][18].

In vivo, the mammalian spermatozoa undergo an intense process during their migration through the female reproductive tract [19]. The passage of sperm through this tract is therefore regulated to ensure only sperms with normal morphology and vital motility will succeed [20][21]. The obstacles in the way of sperms before fertilizing an oocyte are the dynamics of sperm transport, entry, and distribution in the vagina, cervix, uterus, uterotubal junction, sperm storage reservoirs [20], cumulus cells [22][23][24], and zona pellucida [25][26]. Successful fertilization, however, requires high-quality sperm to survive this process [27]. This is defined by a number of factors, including the proportion of viable and motile sperms and their swimming speed, the number of structurally normal and acrosome-intact sperms, the sperms' capacitation ability, and the morphology and relative dimensions of their different components. Discussing these values, however, is out of the scope of this article but could be found in fertility guidelines [28][29]. In this regard, the evaluation and sorting of sperms are essential to the success of assisted reproductive technology (ART) [30][31]. In other words, it is of utmost importance to perform efficient sorting to achieve a sufficient population of morphologically normal and motile sperms with uncompromised DNA integrity and acrosome state [32][33][34].

To mimic the natural sperm selection strategies in ART and to improve its quantity and quality, several advanced methods are developed [12]. These methods are mainly used for sperm selection prior to intra-cytoplasmic spermatozoa injection (ICSI), which was conventionally performed by a clinical embryologist. Some examples of these methods include surface charge selection, hyaluronic acid binding, sperm apoptosis assay, sperm birefringence, intra-cytoplasmic morphologically selected sperm injection (IMSI), motile sperm organelle morphology examination, DNA/chromatin integrity, hypo-osmotic swelling test (HOST), Raman spectroscopy [20][35], and zona-binding sperm selection [25]. This is because the use of microfluidic devices for sperm processing in the past decade has created new opportunities for the field [36].

Microfluidics was adopted for ART purposes in the 2000s [37]. Ever since, it has helped to improve ART results by facilitating different steps, such as embryo culture [38], the trapping and characterization of human oocytes [38][39], in vitro fertilization (IVF) [38], reduction of polyspermic penetration during IVF [40], removal of the zona pellucida from mammalian embryos [41], removal of cumulus from mammalian zygotes [42], sperm monitoring, and finally, sperm sorting [43][44][45][46][47][48][49][50][51].

Microfluidic-based sperm sorting is an important cell-sorting category that is emerging very fast. In fertility studies, as well as infertility treatments, sperm sorting is a crucial step in which viable, motile, and morphologically appropriate sperm cells should be separated from the semen or washed sperm samples for fertilization [3][4][52]. Implementing these steps in a microfluidic platform, as mentioned earlier, enables the completion of various tests in a fast and low-cost fashion, with a lower amount of the target fluid needed and using miniaturized or portable devices.

2. Microfluidic Sperm-Sorting Techniques

Microfluidic platforms for sperm sorting rely on either active or passive methods. In active methods, external stimulators, such as the temperature of chemical gradients or an active fluid flow, perform the sorting, while passive methods rely on the inherent behavior and movement of sperms in the absence of any external stimuli. As part of the design considerations, a microfluidic sorter needs to be safe for sperms such that it will not alter their specifications, such as motility, morphology, DNA integrity, and acrosome. This can be achieved using channels and chambers with sperm-friendly size, length, shape, and coatings. These features can be different in each study according to the specific application and sorting strategy of the designed chip for sperm sorting [1][3]. Similarly, the employed forces and stimuli, such as acoustic waves, chemicals, heat, and electric charges, should not have any negative impact on the sperms, their activities, or the medium surrounding them. Such safety concerns should be taken into consideration also regarding coloring dyes and/or tracking tags used for sperm analysis and imaging purposes inside the microfluidic devices [53][54][55]. On the other hand, as the passive methods are mainly based on the macroscopic morphology and displacement of the sperms, they provide a safer and less invasive sorting approach compared to the active methods. However, they are less capable of benefiting from specific sperm behaviors/characteristics [56][57][58].

2.1. Passive Methods

Passive strategies that were developed for sorting high-quality sperms in microfluidic platforms are summarized in Table 1.

Table 1. Summary of passive strategies applied in microfluidic chips.

Sorting Strategy	Parameter(s)	Advantages	Disadvantages	Significance	Ref.
Geometry	Swimming behavior of sperms, micro-pillar arrays	-Noninvasive -Reduced complexity of structural features -Mimics filtering characteristics of female reproductive tract	-Complicated chip fabrication process due to complex high-aspect-ratio geometry	-Morphology: 5-fold enhancement -Nuclear Maturity: 3-fold enhancement -DNA integrity: 2–4-fold enhancement -Throughput: 99% -Working time: 10 min	[59]
	-Velocity shear gradient -Hydrodynamic profile of fluid micro-confinement	-Simple working procedure	-Complicated chip design and fabrication due to complex high-aspect-ratio geometry	-Retrieval efficiency: 44% increased -Throughput: 80% -Optimized flow rate: 0.7 µL/min	[60]
	-Hydrodynamic profile of fluid within the channel -Fluid flow mechanics -Shear rate butterfly-shape structure	-Mimics the variable width of the junctions within the female reproductive tract -Simple chip design and fabrication	-Accumulation of a large population of sperms in front of the stricture leads to reduced efficiency of sorting highly motile sperms	-Highly progressive motile sperms swim to the fertilized site -Non-motile and slow sperms accumulate in front of the stricture	[61]

Sorting Strategy	Parameter(s)	Advantages	Disadvantages	Significance	Ref.
Rheotaxis	-Rheotactic behavior of sperms -Corrals inside microchannels -Flow rate	-Adding sperm retainer	-Complicated chip fabrication due to complex high-aspect-ratio geometry	-Throughput: 100% -Residence time: 45 min	[62]
	-Fluid flow -Rheotactic behavior of sperms -Gravity	-Automated procedure -Fast sorting -Eliminate the use of additional tools, such as a pump -Simple chip design and fabrication	-Misses some of the potentially high-quality sperms due to the rapid pace	-Optimized delay time between semen injection and suctioning motile sperms: 80 s -Highest figures of motility indexes are mean velocity: 8.94%, motility percentage: 32.58%, motile sperm rate: 21.99%	[63]
	-Fluid velocity inside the channel -Designing a diffuser-type channel	-Simple chip design and fabrication -Performance based on continuity equation in fluid dynamics	-Imprecise collection of sorted sperms in appropriate region	-Throughput: 8.6×10^5 sperms/min -Working time: 10 min -%Motility: 82.24% -Motile sperm rate: 53.10%	[64]
Fluid Flow	-Three different parallel laminar flows -Variable semen flow rate - Ability of sperms to cross streamlines in laminar flow	-Mimic viscous environment of female reproductive tract -Simple chip design and fabrication	-Missing some of potentially high-quality sperms due to time dependency of migration in laminar fluid	-Sperm activity: 95.7%	[65]
	-Diffuser-type channel -Fluid dynamics production -Enabling cross-passage of sperms through laminar flow streamline	-Continuity equation in fluid dynamics	-Complicated chip design and fabrication due to complex high-aspect-ratio geometry	-Motility pattern of more functional sperms: sinusoidal trajectory pattern -DNA integrity: 95% -DNA fragmentation: 18.4–21.9%	[66]

2.2. Active Methods

Recent active strategies that have been applied in microfluidic devices to sort the high-quality and progressive motile sperms are summarized in Table 2.

Table 2. Active strategies that were reported for sperm sorting.

Sorting Strategy	Parameter(s)	Advantages	Disadvantages	Significance	Ref.
Acoustic waves	-Surface acoustic wave -Sperm size -Motility pattern	-External sorting -Precise control of sperm selection process	-Invasive -Need for additional equipment	-Operation time: 50 min -Throughput: 60,000 sperms/cycle -Vitality: 50% -Progressive motility: 60% -DNA integrity: >38% -Swimming velocity: 64%	[67]
	-Bulk acoustic wave -Pressure distribution through the fluid -Addition of polystyrene beads	-Isolates scarce number of sperms from female DNA samples	-Lower power compared to surface acoustic wave -Invasive -Need for additional equipment	-Operation time: 15 min -Particle size of polystyrene beads: equal to sperms -Isolation efficiency: 85%	[68]

Sorting Strategy	Parameter(s)	Advantages	Disadvantages	Significance	Ref.
Chemotaxis	-Progesterone gradient concentration -Sperms' chemoattractant behavior	-Noninvasive -Biomimetic strategy -Flow-free	-Low efficiency	-Sperms chemotactic ratio: 1.41	[69]
	-Ach [1] and rat oviductal fluid gradient concentration -Sperms' chemoattractant behavior	-Uniform gradient -Stationary fluidic environment -Biomimetic strategy -Eliminate rheotactic and chemokinetic behavior of sperms as selection criteria	-Low efficiency	-Improved number of entered sperms by increasing ACh concentration: 20% -Sperm population with chemotactic behavior in ACh-rich environment: 8.5% -Sperm population with chemotactic behavior in oviductal fluidic environment: 6.6%	[70]
Chemotaxis and thermotaxis	-ACh gradient concentration -Temperature gradient -Sperms' chemoattractant and thermoattractant behavior	-Flow-free -Biomimetic strategy	-Complicated chip design and fabrication due to complex high-aspect-ratio geometry -Need of additional structural features	-Optimized temperature gradient: 0.154 °C/mm from 35 to 37 °C	[71]

[1] ACh: acetylcholine.

3. Conclusions and Future Directions

Microfluidic-based devices have shown promising results for sorting spermatozoa using various on-chip mechanical and chemical stimuli. Applying fluid mechanics features at the microscale to manipulate the efficient movement of only motile sperms is the core of such approaches. Both stimuli- and non-stimuli- (mechanical) based methods have their advantages and disadvantages. This is why the stimuli should be selected in a way that would not harm the sperms. These conditions are well explained in the literature and therefore should be used as a guideline in selecting the stimuli. Moreover, active-based sorters need a module to apply the stimulant. This makes the design more complicated due to the complex high-aspect-ratio geometry in the microstructures with micropillars or microwalls that affect the size, price, and portability of the device. Those devices relying on chemotaxis and thermotaxis, especially, need reservoirs for the reagents and special training to use them. Passive methods, on the other hand, are less complicated in this regard but, at the same time, not as efficient as active methods and therefore have limited potential applications for sperm sorting. Most PoC devices are designed to benefit from a phone camera as an imaging system to facilitate the design. Therefore, taking all these into account, the final decision on which technique to use should be determined based on the application and considering the circumstance.

Considering the above-mentioned promising results, such labs-on-chips are expected to soon become more commonly used in infertility treatment centers around the world. However, they are expected to evolve in two main aspects. One is the application of more complex flow manipulation strategies through implementing two or more sorting systems in order to improve the quality and specificity of the process. This can be achieved, for instance, through the simultaneous application of acoustic waves and chemical attraction methods. Such chips would require a precise design to avoid any possible damage to the sperm. However, such modifications might increase the overall cost of the tool but would allow for improving the sorting efficacy. Exploring new stimulants, such as electrical stimulants, and the use of nanoparticles are other options.

An ideal such lab-on-a-chip should be capable of efficient sorting, along with real-time monitoring and quality control of the IVF steps in an automated manner. The need for automation and serial sample manipulation while reducing the number of preparation steps and the cost is therefore another aspect to be addressed in the future. Such improvements can be achieved through combining the sorting, oocyte culturing, and conception steps all in a single or interconnected chip. On-chip flow manipulations can be controlled using programmable on-chip micropumps and microvalves [72][73]. In addition, artificial intelligence and machine learning [74] have a high potential to be used in such chips or for analysis purposes.

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