Microfluidic Sperm Selection

53

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Key Points

- Sperm cells show tendency to swim and accumulate close to surfaces instead of spreading in a tridimensional space. This behavior can be exploited using microchannels that increase the processing surface.
- The pattern of motility and migration of spermatozoa in viscous media differs substantially from the pattern exhibited in nonviscous media. This feature can be exploited by the use of culture media with different viscosities or even generating viscosity gradients.
- Sperm exhibit positive rheotaxis, which is the tendency in swimming against a liquid flow. This feature can be explored with the use of constant flow streams or even generating flow gradients.
- The classic configuration of a microfluidic chip consists of an inlet reservoir, a connecting channel, and an outlet reservoir. The liquid volume in each reservoir will define whether there will be a flow or not, and if so, what is the direction of flow. Scaling, orientation, and combination of these basic components can lead to a variety of systems with different selective characteristics.
- The selective approach of microfluidic devices resembles natural sperm selection processes, indirectly attesting the functionality of the male gamete.

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53.1 Introduction

Among the millions of sperm present in the ejaculate, only hundreds or even dozens can reach the ampulla or the site of fertilization [1]. The task of accomplishing its purpose is hindered to the ones without the proper attributes by selection mechanisms of the female reproductive tract.

Several studies report the existence of different populations of spermatozoa within the same ejaculate, which have different characteristics in terms of morphology, motility, chromatin integrity, mitochondrial status, and others [2–6]. This phenotypic heterogeneity might be caused by genetic variability, epigenetics, disturbed spermatogenesis, altered epididymal sperm transit time, etc. [7]. In fact, this diversification of sperm function and structure may lead to distinct performances depending on the obstacles to be overcome.

In the female reproductive tract, these challenges translate into migration through viscous media (cervical mucus), migration through confined spaces (uterine villi), and countercurrent migration (against the flow of tubal fluid) [8–10]. Among the different populations that compose the ejaculate, only the ones with specific phenotypical features will be able to overcome the various hurdles and will have the "opportunity" to fertilize the oocyte.

Of the characteristics found in spermatozoa collected in fallopian tubes after mating in various studied species, three stand out: progressive motility, normal morphology, and chromatin structure [11]. Many selection mechanisms acting together in the female reproductive tract are responsible for this selection, eliminating the spermatozoa that do not exhibit the necessary attributes to the oocyte fertilization and further embryonic development [1].

The viscosity of the cervical mucus imposes itself as an obstacle to the morphologically abnormal spermatozoa (immature sperm), presenting residual cytoplasm and poor DNA packaging. In result, such featured gametes will be eliminated right on the beginning of the path [12]. Additionally, the migratory effort throughout the female reproductive tract appears as an obstacle to those spermato-

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zoa that have some degree of mitochondrial damage due to apoptotic processes linked to senescence or due to reactive oxygen species (ROS) attack. Such gametes will not have enough resistance to complete the path, even if exhibiting normal morphology [13–18]. Therefore, in a simple way, we can infer that normal morphology attests the spermatogenesis efficiency, whereas vigorous motility attests the functional excellence of spermatozoa. Thereby, the natural sperm selection processes can be compared to the "Ironman" Championship, where different challenges are imposed (swimming, cycling, running). The ones who are able to complete the course will be apt to fertilize the oocyte. The success now will depend on whether they are in the correct uterine tube in the right moment or not. The spermatozoa remained along the way will never have this chance. It is not an infallible process, but it is an effective method to select the best characteristics from such heterogeneous semen samples.

53.2 Sperm Characteristics that Can Be Used as Selection Factors

The spermatozoon is a peculiar cell: it is the only flagellated cell in humans and carries its highly compacted genetic material confined into a volume that is typically 10% of a somatic cell nucleus [19]. This uncommon cell shows particular characteristics that can be used as key points in the selection process.

53.2.1 Migration in Space-Constricted Environment

Sperm cells show tendency to swim and accumulate close to surfaces [20]. Instead of spreading in a tridimensional space, these cells follow a channel border or the surface of a glass slide [21]. It is estimated that swimming following the surface or border is approximately 50% faster than swimming in the channel center [22]. This confers benefit in confined regions of the reproductive tract or in microchannels.

53.2.2 Migration Through Viscous Fluids

The pattern of motility and migration of spermatozoa in viscous media differs substantially from the pattern exhibited in nonviscous media, such as those used in assisted reproduction technologies (ART) [10]. In low viscous media, the swimming pattern is characterized by the rotation of the flagellum in its longitudinal axis and consequent head rolling (rolling mode) [23]. This tail movement produces a cone-shaped helical pattern with a cross section many times larger than the diameter of the sperm head [9]. That is, morphology of the head does not influence the sperm hydrodynamic behavior in low viscous media. However, in viscous media, the sperm angular speed (rotation along its longitudinal axis) decreases, resulting in a planar movement of the tail [24]. This makes the morphology of the head and midpiece much more relevant in hydrodynamic terms.

53.2.3 Positive Rheotaxis

Rheotaxis is the tendency of a cell to orient its movement against or in favor of the fluid flow that surrounds it. Positive rheotaxis is the tendency to swim against this flow of fluid [9]. Several studies describe this behavior as being the main factor that guides spermatozoa to the oocyte [25–28]. The main argument is based upon the evidence that there is an increase in fluid flow from the uterine tubes after coitus. This flow would help transporting the oocyte to the uterus and guide the spermatozoa toward the oocyte [29]. Countercurrent swimming demands vigorous and constant motility.

53.2.4 Thermotaxis

Thermotaxis is the tendency of modifying the direction of movement following a temperature gradient [30]. Like the rheotaxis, it is also considered a sperm orientating factor that manifests in long distances. Thermotaxis manifests following the temperature gradient established at the uterus-tubal junction during ovulation [31, 32]. It is believed that only spermatozoa that suffered sperm capacitation are responsive to temperature changes [33].

53.2.5 Chemotaxis

Phenomenon of chemotaxis has been observed for many years in animals presenting external fertilization. It is believed that chemoattractants secreted by oocytes are able to alter the pattern of spermatozoon tail beating orientating it toward the oocyte [34]. However, in mammals, their role in sperm orientation is still controversial. Even though the presence of chemoattractant substances in the follicular fluid was confirmed, uterine contractions and ciliary currents would be able to disrupt the gradients, which would make it not viable for long-distance orientation [35]. It is believed that chemotaxis may be relevant as a short-distance mechanism only.

53.3 Current Sperm-Sorting Technologies

The intracytoplasmic sperm injection (ICSI) revolutionized the treatment of male-factor infertility. Nevertheless, the use of this technique raises concerns about the possibility of inadvertent selection of a spermatozoon containing DNA damage [36–38]. ICSI eliminates all natural selection barriers, since the spermatozoon is directly injected into the oocyte cytoplasm [39]. In practice, it comes down to a subjective "choosing" process. Once polyvinylpyrrolidone (PVP) prevents the analysis of motility, morphological analysis is the only remaining resource to the embryologist. While visual inspection permits a trained embryologist to identify immature spermatozoa, the damage in sperm DNA itself does not result in any morphologic changes, making its identification nearly impossible [36]. The success of sperm selection will depend not only on the technique used but also on the quality of the ejaculates.

As seen before, ejaculate is a heterogeneous mixture of sperm from which we must pick some individuals. Therefore, previous seminal processing is crucial to ICSI, not only for the removal of seminal plasma but also for the removal of spermatozoa that do not show those fundamental attributes: superior morphology, vigorous motility, and intact chromatin [40]. The more efficient the selection, the lower the risk of inadvertent injection of a nonfunctional gamete [37].

Sperm wash is the simplest seminal processing technique that has been utilized in the field of assisted reproductive technologies (ART). It may not be considered a sperm selection technique, since there is no removing of inadequate spermatozoa, but seminal plasma removing only. This technique is used lone for the processing of poor seminal samples [41].

Discontinuous density gradient (DG) highlights among the most commonly used sperm selection techniques. It is based on the sedimentation capacity of the sperm through layers of culture medium with different densities, after centrifugation [40]. It is efficient for the removal of immature spermatozoa, which are less dense, but does not show the same performance in the removal of spermatozoa with DNA fragmentation. The migratory effort is minimal.

Swim-up is another technique widely used in clinical practice. In this method, motile sperm migrate from a sample deposited on the bottom of a conical tube into an overlaying medium [42]. This approach explores sperm motility, but over short distances [43]. The migration occurs mainly through the liquid column, since the small surface area does not allow sperm accumulation by the near-wall swimming effect [21]. Swim-up is efficient in the removal of spermatozoa with DNA damage; however, it does not present the same performance in the removal of abnormally chromatin-condensed spermatozoa [40]. This technique recovers relatively low numbers due to two main reasons: the small surface area for migration and the entrapment of motile spermatozoa in the lower layers of the sample. For this reason, this technique is inadequate for the processing of poor samples and is still the standard method for patients with normozoospermia and female infertility [40].

53.4 Microfluidics

A new approach to innovative techniques for the selection of functional spermatozoa is the use of microfluidics, which is the manipulation of tiny volumes of liquid. Microfluidic devices have microchannels that explore the tendency of sperm to migrate along surfaces [20, 44]. In this way, the possibility of subjecting the male gametes to several selection factors in a controlled environment is created. Recent papers describing microfluidic sperm selection devices, most of the times, use distinct classification focusing on the bioengineering characteristics of these devices [29, 41, 45]. We will be using an approach which focuses on the selection factors of each of these devices, that is, the characteristics that distinguish the sperm population meant to be isolated. Some devices use distinct mechanisms to isolate the same sperm population.

The classic configuration of a microfluidic chip consists of an inlet reservoir, a connecting channel, and an outlet reservoir. If the system works equally in volume for both reservoirs, there will be no liquid flow in the channel. If the inlet reservoir volume is higher than in the outlet reservoir, there will be a directional flow. Once the volume goes higher in the outlet reservoir than in the inlet reservoir, there will be an inverted flow (Fig. 53.1a–c). The demanded migratory effort is minimum in directional flow systems, as the flow "pushes" spermatozoa toward the outlet reservoir. In no flow systems, however, there is an important migratory effort, as spermatozoa must swim during the whole course toward the outlet reservoir. Finally, in inverted flow systems, the migratory effort is even greater, because the migration will occur against liquid flow. These basic characteristics can be multiplied or combined in the search for an efficient device.

53.4.1 Chemotaxis and Thermotaxis

Sperm capacitation is a series of structural and functional modifications that give spermatozoa the ability to fertilize the oocyte. Human capacitated spermatozoa appear to behave similarly when exposed to a temperature gradient or to a chemoattractant gradient, directing the swim toward the origin of the stimulus, without changes of direction.

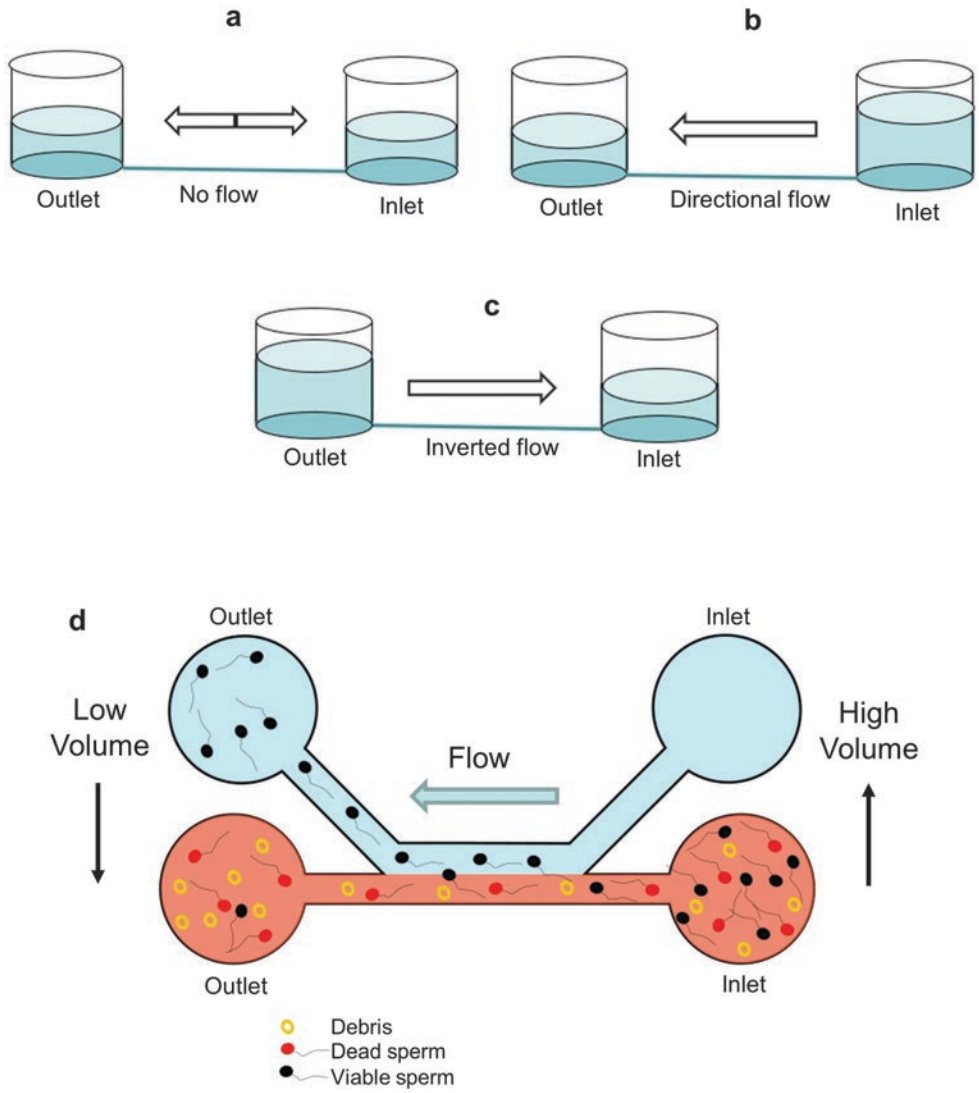

Fig. 53.1 Basic fluid dynamics in a microfluidic sperm selection chip. (**a**) No flow system. The volume of liquid is the same in both reservoirs. There is no flow in the channel. (**b**) Directional flow system. The volume of liquid in the inlet reservoir is higher than in the outlet reservoir. There is liquid flow in the channel from the inlet reservoir to the outlet reservoir. (**c**) Inverted flow system. The volume of liquid in the outlet reservoir is higher than in the inlet reservoir. There is liquid flow in the channel from the outlet reservoir to the inlet reservoir. (**d**) Microfluidic sperm selection device developed by Cho et al. [51]. It is a directional flow system containing two inlet reservoirs, two outlet reservoirs, and a common main channel. Motile sperm capable of changing from one stream to another are carried to the respective outlet reservoir. (**d**: Adapted with permission from Cho et al. [51]. Copyright (2003) American Chemical Society)

Microchannels can help guiding the gametes, increasing selection efficiency. Thus, only capacitated spermatozoa possessing the specific receptors would be able to migrate toward the origin of the stimulus, where they will accumulate. Microfluidic devices that explore chemotaxis [46–48] and thermotaxis [49, 50] are still experimental concerning to human sperm selection.

53.4.2 Short-Distance Migration

This strategy resembles that used in swim-up. The focus is on obtaining motile spermatozoa. It aims to select spermatozoa capable of swimming short distances, separating them from debris, immotile spermatozoa, and seminal plasma.

Cho et al. used a passively driven integrated microfluidic system to separate motile sperm from raw semen samples [51]. Due to a phenomenon known in the field of microfluidics as "laminar flow," the liquid from two parallel flows present in the main channel and coming from distinct reservoirs do not mix (Fig. 53.1d). This allows motile sperm to migrate from the stream containing the seminal sample to the stream of sperm-sorting medium [51, 52]. A strong flow pushes the spermatozoa to a specific reservoir, separating them from the debris and dead cells present in the original stream (Fig. 53.1d). The liquid streams are generated by a pressure difference between inlet reservoirs and outlet reservoirs. The volume of liquid in inlet reservoirs is higher than in outlet reservoirs causing the difference in pressure [53, 54]. The spermatozoa recovered show higher vitality, better morphology, and less DNA fragmentation when compared to the original sample [55].

Seo et al. developed a similar system; they used rheotaxis to the initial separation of spermatozoa, though [56]. Motile spermatozoa able to migrate outside from the inlet reservoir are directed to swim against a weak flow until reaching a

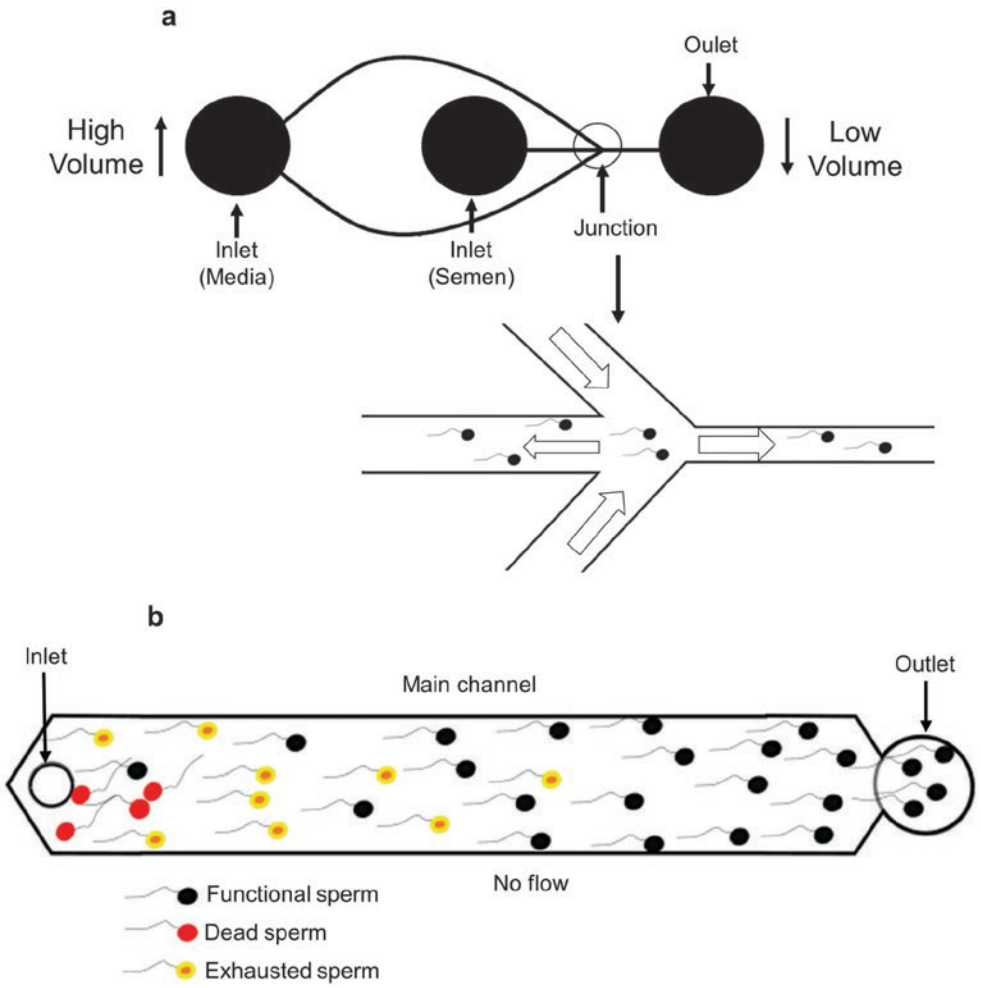

Fig. 53.2 (**a**) Microfluidic sperm selection device developed by Seo et al. [56]. It is a directional flow system containing two inlet reservoirs and one outlet reservoir. The main flow occurs between the media inlet reservoir and the outlet reservoir. There is a weak secondary flow toward the semen inlet reservoir that stimulates positive rheotaxis behavior. The spermatozoa that reach the junction are pushed toward the outlet reservoir. (**b**) Microfluidic sperm selection device developed by Tasoglu et al. [59]. This is a no flow system. Resilient sperm are able to migrate from the inlet reservoir to the outlet reservoir (functional sperm). Sperm unable to migrate for long distances due to structural or physiological impairments do not reach the outlet reservoir (exhausted sperm). (**a**: Adapted from Seo et al. [56]. With permission from Springer Nature)

junction. Spermatozoa are dragged by a strong flow to the outlet reservoir from this point (Fig. 53.2a).

The migratory effort is relatively small for both techniques, enough only to leave the semen sample. The transporting to the outlet reservoir is made by means of a strong flow of liquid.

53.4.3 Resilience

Although the role of mitochondria in sperm function is still a matter of debate, several studies report that sperm motility is closely related to mitochondrial membrane potential (MMP) [4, 18]. Besides, there MMP is correlated with DNA integrity as well [16, 17]. Therefore, we may suppose that spermatozoa able to high migratory efforts hold intact DNA [11]. Perhaps that is where the key for the success of natural processes in obtaining high-quality gametes relies on.

Spermatozoa migration in "extended drops" had already been used mainly for poor semen samples, but there was no accurate estimate of how far the gamete should go for its functionality to become attested [57, 58]. The use of microfluidic devices enabled this analysis. Comparing spermatozoa migration through channels of different lengths, Tasoglu et al. determined that a 2 cm migration is enough to obtain functional human spermatozoa [59]. According to the authors, sperm exhaustion is an important phenomenon in microfluidic sperm selection.

Based on the experimental data, a microfluidic device for clinical use was developed [60]. The device displays the classic configuration: an inlet reservoir, a connection channel, and an outlet reservoir. The system is filled with low viscosity culture medium. The spermatozoa must actively swim from the inlet reservoir through the main channel to the outlet reservoir, where they are collected. There is no flow and it is a considerable migratory effort. The spermatozoa that show low resistance to migration stand in the way and are eliminated (Fig. 53.2b). A recently published study shows that the spermatozoa recovered with this type of device exhibit greater vitality, better morphology, and less DNA damage compared to density gradient centrifugation and swim-up [61].

Nosrati et al. developed a device that expands the layout inlet/channel/outlet. It is a platform containing 500 channels

in a radial arrangement filled with a viscoelastic medium [62]. Yet again, there is no flow. The viscosity of the surrounding medium makes the migration even more difficult (Fig. 53.3). The device was tested for raw semen sample processing, showing up an 89% improvement in vitality and 80% improvement in sperm DNA integrity.

De Martin et al. developed the positive rheotaxis extended drop (PRED) which also exhibits the classical configuration. In this inverted flow system, there is a difference of hydrostatic pressure between the reservoirs, generating a flow of liquid from the outlet reservoir toward the inlet reservoir [63]. Besides that, polyvinylpyrrolidone (PVP) deposited in the distal end of outlet reservoir generates a viscosity gradient. Thereby, spermatozoa placed in the inlet reservoir must leave the semen sample (short-distance migration), swim against a fluid flow through the connecting channel (positive rheotaxis), and migrate against a viscosity gradient until reaching the outlet reservoir where they will be captured (migration through viscous media). The circuit is set manually in an ICSI dish (Fig. 53.4a, b). Spermatozoa that were able to reach the distal end of the outlet reservoir will be collected with an ICSI needle and injected in the oocyte. Therefore, the PRED dish tries to mimic natural obstacles faced by the spermatozoa such as migration in viscous media, migration in confined environments, and countercurrent migration. The device was able to reduce uncondensed chromatin spermatozoa to nearly 1%, processing raw semen samples [63].

Wu et al. described the flowing upstream sperm sorting (FUSS) that is basically a directional flow system that works

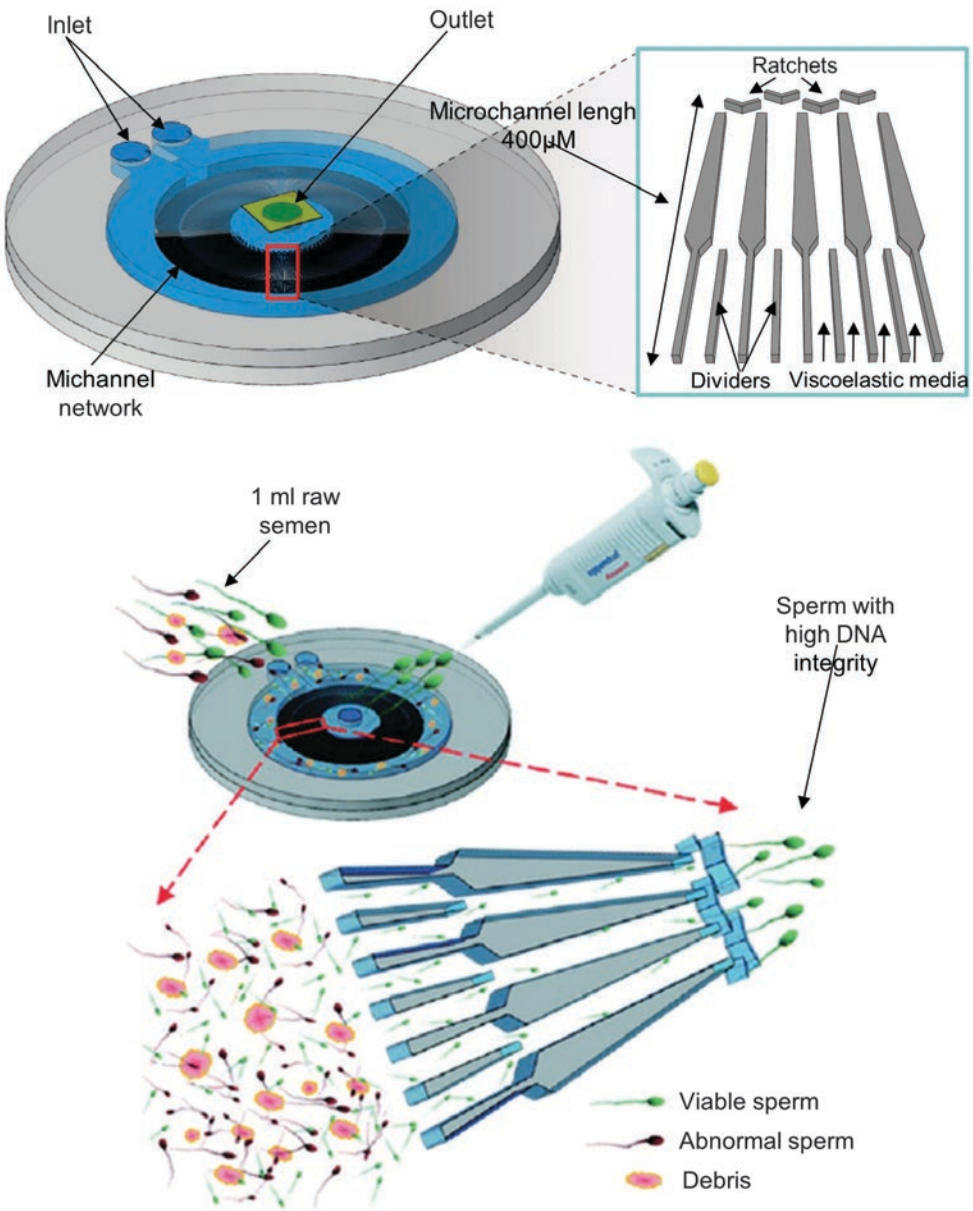

Fig. 53.3 Microfluidic sperm selection device developed by Nosrati et al. [62]. It consists of 500 parallel channels in a radial array filled with viscoelastic media. Motile sperm guided by dividers migrate through the channels toward the central outlet reservoir (viable sperm). Sperm unable to migrate for long distances due to structural or physiological impairments do not reach the outlet reservoir (abnormal sperm). (Reprinted from Nosrati et al. [29]. with permission from Springer Nature)

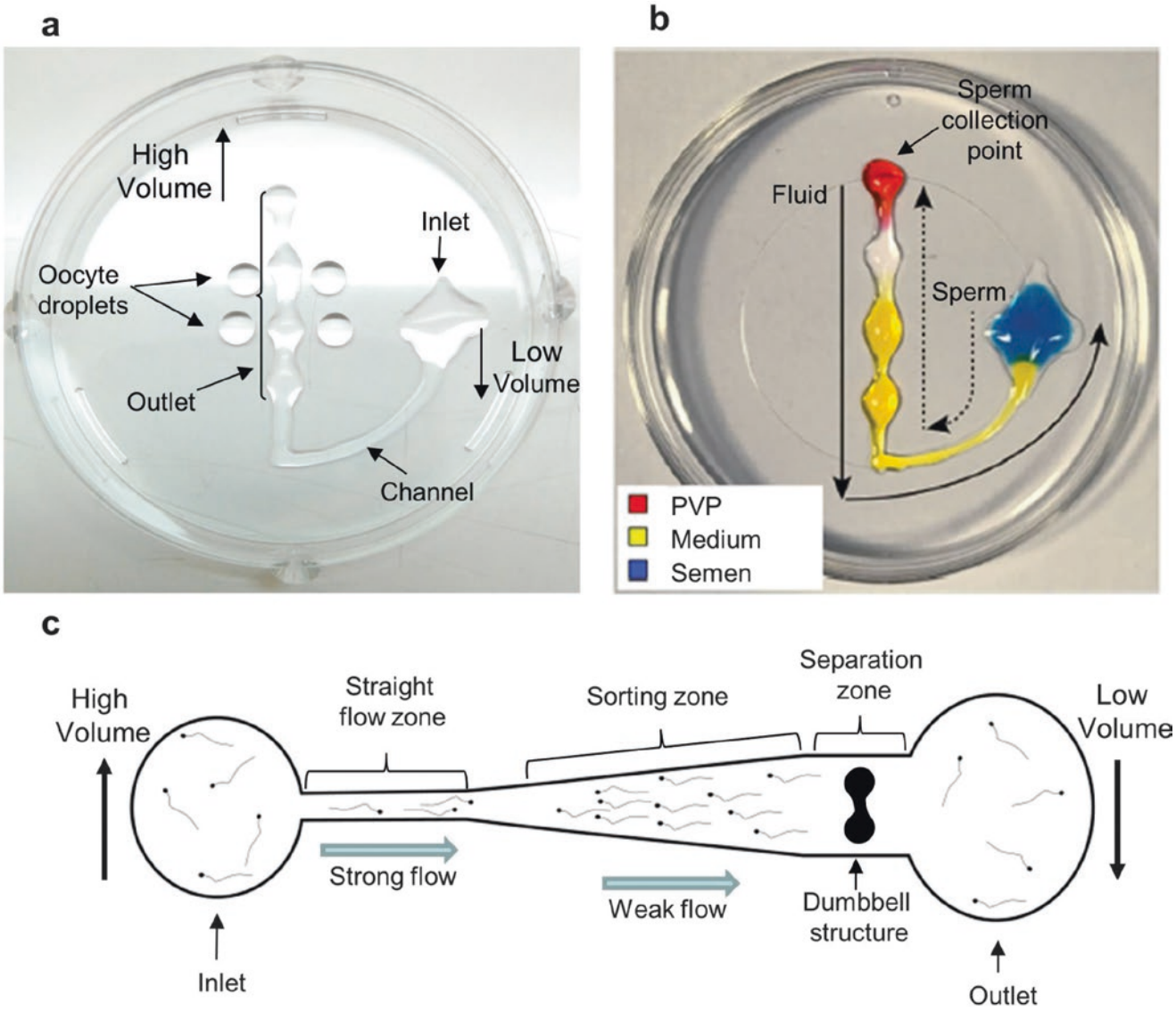

Fig. 53.4 Microfluidic sperm selection device developed by De Martin et al. [63]. (**a**) It is an inverted flow system containing one inlet reservoir, a connecting channel, and a long outlet reservoir. PVP is added at the distal end of the outlet reservoir. The system is set on an ICSI dish. (**b**) There is liquid flow through the channel from the outlet reservoir. This flow of culture medium prevents diffusion of the semen sample from the inlet reservoir. The spermatozoa deposited in the inlet reservoir should swim against the flow through the channel and against a viscosity gradient until reaching the distal end of the outlet reservoir where they will be captured. **(c)** Microfluidic sperm selection device developed by Wu et al. [64]. It is a directional flow system with a peculiar characteristic. The channel intermediate sector shows progressively higher widths which generates a velocity gradient allowing the positive rheotaxis behavior. Motile spermatozoa that remain swimming against the flow are "trapped" in this sector, while immotile sperm and debris are carried out to the outlet reservoir by the same flow. (**a**, **b**: Reprinted from De Martin et al. [63]. With permission from Springer Nature)

as an inverted flow system [64]. The system shows the classic configuration (inlet/channel/outlet) with the flow being directed from the inlet to the outlet. The spermatozoa initially deposited in the inlet are drawn out by a strong flow toward the outlet at the first segment of the circuit (straight-flow zone). The channel has an enlargement in its intermediate part (diffuser-type sperm sorter) that slows down the flow, allowing the spermatozoa to swim against the fluid stream (positive rheotaxis). As the channel increases its width, the flow velocity decreases, and this allows to differentiate sperm with different motilities. Debris, dead cells, and immotile sperm are carried by the flow to the outlet. Motile sperm are collected in the diffuser channel (Fig. 53.4c). The system was able to recover enriched samples containing 80% viable sperm, processing batches of ~ 200,000 spermatozoa, with an estimated time from 5 to 15 minutes.

53.5 Future Perspectives

The strategy of mimicking the natural selection processes is promising in obtaining functional spermatozoa. Some microfluidic devices are already available in the market, and more comprehensive clinical trials evaluating their effectiveness are on the way. One of the factors hindering the adoption of these devices in daily clinical practice is the relatively high cost. This is due to the use of materials and production processes suitable for laboratory tests but unsuitable for mass production. The adoption of industry-friendly materials and processes can help reduce manufacturing costs and consequently the prices.

Microfluidics allows unprecedented control of the environment that surrounds sperm. Thus, the viscosity, the flow velocity, the distance to be traveled, and the time spent in each step can be manipulated. With the evolution of microfluidic technology, the selection of functional spermatozoa may be more efficient than that exhibited in the female reproductive tract. It would be possible to obtain spermatozoa with intact DNA even from poor semen samples. In addition, the use of microfluidic chips in clinical practice will aid in the standardization of processes improving the treatment of male factor infertility.

53.6 Conclusion

The inadvertent injection of a spermatozoon containing DNA damage is a growing concern, and sperm selection techniques may potentially prevent this from happening. It would be ideal if we could access the chromatin status of each spermatozoon prior to injection, but this is not feasible without destroying it or compromising its functionality. Probably, the most effective approach would be to access male gamete functionality indirectly. Thus, the sperm would be tested not for what it is but for what it can do. The challenge is to find out which is the least challenge or effort able to select functional spermatozoa. Therefore, microfluidics may be the ideal tool in the aim of reaching this purpose due to its capacity of control and precision.

53.7 Review Criteria

A careful investigation of all the articles related to the use of microfluidics in the selection of functional spermatozoa evaluating articles published until October of 2018 was carried out. The search engines Google Scholar, PubMed, Science Direct, and MEDLINE were used. The search was limited to studies published in English. Searches were performed using keywords such as “microfluidics,” “microfluidic technologies,” “microfluidic chip,” “sperm sorting,” “sperm selection,” “motile sperm,” “semen,” “male,” “infertility,” “in vitro fertilization,” and “sperm DNA fragmentation.”

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