Microfluidic devices for the study of sperm migration

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ABSTRACT: Microfluidics technology offers us an opportunity to model the biophysical and biochemical environments encountered by sperm moving through the female reproductive tract and, at the same time, to study sperm swimming dynamics at a quantitative level. In humans, coitus results in the deposition of sperm in the vagina at the entrance to the cervix. Consequently, sperm must swim or be drawn through the cervix, uterus, uterotubal junction and oviductal isthmus to reach the ampulla of the oviduct. Only a very small percentage of inseminated sperm reach the ampulla in the periovulatory period, indicating that strong selection pressures act on sperm during migration. A better understanding of how sperm interact with the female tract would inspire improvements in diagnosis of fertility problems and development of novel-assisted reproductive technologies that minimize damage to sperm and mimic natural selection pressures on sperm.

Key words: microfluidics / sperm motility / sperm migration / cervix / uterus / oviduct / fallopian tube / rheotaxis / chemotaxis

Introduction

We know very little about the dynamics of sperm migration through the mammalian female reproductive tract, particularly of the human tract. Of the millions of human sperm inseminated, only tens to hundreds of sperm reach the ampulla of the oviduct, where fertilization occurs, during the periovulatory period (Williams et al., 1993). The arrival at the egg of but a small fraction of inseminated sperm indicates that there are strong selection pressures acting on sperm at various times during their movement through the female tract.

In humans, natural coitus results in the deposition of sperm in the vagina very near the entrance to the cervix (Sobrero and Macleod, 1962). Subsequently, sperm must swim or be drawn through the cervix, uterus, uterotubal junction (UTJ) and oviductal isthmus to reach the egg in the oviductal ampulla (Fig. 1). Each of these segments of the female tract presents sperm with different structural, fluidic, ionic and molecular environments; therefore, the process of sperm migration is complex.

It is important to understand more deeply the process of human sperm migration through the female tract in order to improve diagnosis of the causes of infertility/subfertility. It has been estimated that 10–30% of couples seeking treatment for infertility receive a diagnosis of unexplained infertility (Gunn and Bates, 2016). Recent developments in microfluidics technology provide novel means to elucidate specific selection pressures on sperm and to apply them toward diagnosing male fertility problems. Microfluidics technology enables us to make models of the anatomy of the compartments through which sperm must travel, with reproducibility down to the level of microns. It also enables us to model the physical properties of fluids and the speeds of fluid flows encountered by sperm.

Microfluidics additionally offers the potential to provide a more efficient and physiological means to select sperm for artificial reproductive technology (ART). The two most common methods currently used to prepare human sperm, density gradient centrifugation and swim-up (Gunn and Bates, 2016), each involve the use of centrifugation, which exposes sperm to a level of reactive oxygen species that can damage DNA (Twigg et al., 1998). These techniques are also difficult to apply when the patient produces only low numbers of sperm with apparent normal motility and morphology.

This review is focused on what is currently known about human sperm migration and microfluidic applications for human reproduction. Work on other species is discussed when it illuminates our understanding of what may happen in humans or may be applied to human infertility diagnosis or treatment. Recent reviews are available on sperm migration in other mammalian species (Okabe, 2015; Suarez, 2015, 2016).

Human sperm migration through the female tract

A number of factors have been implicated in affecting sperm migration through the female reproductive tract. Physical factors include wall effects, fluid flow and fluid viscoelasticity. Sperm are also capable of responding to temperature gradients, if they exist (Boryshpolets et al., 2015). Molecular factors include chemotactic gradients and interactions...

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of ligands on sperm with receptors on the walls of the female tract. Here, we will consider the factors that affect sperm migration in each compartment of the female reproductive tract, focusing on what is known about human sperm.

The cervix

Human semen is deposited during coitus in the cranial vagina at the entrance to the cervix (Fig. 1). Sperm immediately begin to enter the cervical canal (Sobrero and Macleod, 1962). If human sperm migrate directly through the cervix by swimming, they could reach the uterus in about 15 min, given an average speed of human sperm in estrous cervical mucus of 35 \( \mu \text{m/s} \) (Katz et al., 1978) and a cervical length of 3 cm.

Little is known about how human sperm migrate through the cervix; however, investigations with a bovine model suggest that sperm could be guided through the cervix by entering microgrooves in the walls of the endocervical canal (Fig. 2E). Like humans, bulls inseminate cows by depositing semen in the vagina near the entrance to the cervix. In cows, microgrooves have been traced through the length of the endocervical canal; this provides pathways for sperm, to bring them all the way from the vagina to the uterus (Mullins and Saacke, 1989). Transmission electron microscopy of sections of bovine cervix prepared after mating revealed cilia within the microgrooves that were oriented to beat toward the vagina (Mullins and Saacke, 1989). Sperm were plentiful in the microgrooves and were oriented toward the uterus, against the gentle currents that would have been produced by the cilia. Gentle currents, roughly 15–100 \( \mu \text{m/s} \), cause bull sperm to orient their swimming into a flow, whereas sperm are swept downstream by stronger flows (Tung et al., 2015b). By entering the microgrooves, sperm can avoid a stronger outward fluid flow in the main endocervical canal, a flow that is thought to prevent microbial pathogens from ascending the tract. A microfluidics model of microgrooves set in a larger channel (described in detail in Fig. 2A–D) demonstrated that bull sperm access microgrooves and orient upstream, whereas the sexually transmitted, flagellated pathogen *Tritrichomonas foetus* does not (Tung et al., 2015b).

The microgroove mechanism of cervical passage of sperm has not been investigated in humans; however, the microfluidics model could easily be used to examine if human sperm behave similarly to bull sperm and access microgrooves. The similarity in size, shape and swimming patterns of human and bull sperm strongly indicates that human sperm would do so. Also, although published images suggest that similar microgrooves exist in human cervices (Kessel and Kardon, 1979), the presence and paths of microgrooves remain to be studied in humans.

It has also been proposed that human sperm are drawn through the cervix by cranially directed waves of contractions of the uterine wall. Ultrasonography revealed that peristaltic contractions, which travel cranially and caudally early in the follicular phase of the menstrual cycle, become dominated by cranially directed contractions in the late follicular phase (Kunz et al., 1996; de Ziegler et al., 2001). Deposition of technetium radiolabelled spheres of albumin in the cranial vagina led within minutes to detection of radioactivity in the oviduct ipsilateral to the ovulating follicle (Kunz et al., 1996). The detection of cranially directed contractile waves seems contradictory to the model of sperm swimming through the cervix in microgrooves. Insufficient information is available to enable us to know which mechanism is responsible for bringing fertile sperm to the site of fertilization. It is possible that both mechanisms act to move sperm toward the uterus, although likely at different times. We argue that the microgroove mechanism would be more advantageous to the female, because it allows selection of vigorously motile sperm and does not promote ascension of pathogens through the tract. Also, such rapid transport of rabbit sperm to the site of fertilization has been demonstrated to damage nearly all of the sperm that reach the oviduct (Overstreet and Cooper, 1978).

The uterus

Movement of live, swimming human sperm in the uterus has not yet been studied directly. It should be noted that, in the non-pregnant...
woman, the uterine cavity is only about the same length as the endocervical canal and the volume of the uterine cavity is only 80–180 μl (Cassilen, 1986). This indicates that there is little open space in the uterus and that the sperm do not have an appreciable distance to travel through the uterus to the UTJ. If uterine fluid is not as viscous as cervical mucus, sperm might be able to move through the uterus in less than 15 min. The uterine opening of the cervix and the left and right cervical mucus, sperm might be able to move through the uterus in less than 15 min. The uterine opening of the cervix and the left and right cervical mucus might be able to move through the uterus in less than 15 min. The uterine opening of the cervix and the left and right cervical mucus might be able to move through the uterus in less than 15 min.

Figure 2 A microfluidic device for modeling the microgrooves in the bovine cervix, reprinted with permission from Tung et al. (2014). (A) A silicon polymer, poly(dimethyl siloxane)(PDMS) device bonded onto a PDMS coated glass slide (1.5’’ × 3’’), with a port on the right for fluid flow input. (B) Close-up view of the six sets of channels in the device, four with grooves (G) and four without (F). (C) Close-up view of a grooved channel (H120 μm × L300 μm) with nine microgrooves (W20 μm × H20 μm). (D) A three-dimensional diagram of a grooved channel. Note that the microgrooves are in the ceiling of the channel, so that sperm cannot accidentally access them by sinking (not shown to scale). (E) Frozen tissue section of a small portion of the wall lining the endocervical canal of a bovine cervix, stained with periodic acid–Schiff/hematoxylin. Microgrooves are seen in cross section along the wall and one of the microgrooves is indicated by arrows (scale bar = 40 μm). Note that frozen sections were used because standard fixation and dehydration of tissue would have artificially enlarged the microgrooves. Detailed methods are described in Suarez et al. (1997).

In addition to the physical factors that could facilitate sperm passage through the UTJ into the oviduct, molecular interactions might play a role as well. It has been established in mice, particularly by using gene manipulation, that specific surface proteins are required on sperm to enable them to enter the oviduct from the uterus (Okabe, 2015). As yet, the mechanism by which the proteins enable sperm to pass through the UTJ is not clearly understood; furthermore, the need for specific sperm surface proteins has not yet been established for humans.

The UTJ

The process of sperm transport through the UTJ is also not well understood. The opening of the UTJ into the oviducts in humans resembles a funnel when viewed from the uterine cavity, and the lining of the UTJ has a simpler topography than that of most non-primate mammals (Fig. 3A) (Hafez and Black, 1969; Brosens and Gordon, 1990). Possibly, the relatively simple funnel shape of the human UTJ serves to guide sperm into the oviduct.

Once human sperm enter the UTJ, they encounter a gentle, ciliary-driven fluid flow from the oviduct into the uterus (Gaddum-Rosse et al., 1973) that could orient the swimming of sperm into the oviduct. Upstream swimming of sperm, known as rheotaxis, has been demonstrated to occur in human sperm (Kantsler et al., 2014; Bukatin et al., 2015; Zhang et al., 2016).

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The oviduct

Molecular interactions between sperm and the female tract have been implicated in holding sperm in a storage reservoir in the lower oviduct in mammals (Miller, 2015; Suarez, 2016). There is evidence in some species that this storage reservoir serves not only to maintain the viability of sperm until ovulation but also to limit polyspermic fertilization by gradually releasing sperm to ascend the oviduct to meet oocytes (Hunter, 1972, 1973; Muro et al., 2016).

With respect to holding sperm in the storage reservoir, neither oviductal receptors nor sperm ligands have been definitively identified in humans, but there is evidence that the glycoprotein beta-defensin 126 (DEFB126) on the surface of macaque monkey sperm mediates attachment to oviducal epithelium. Shedding of DEFB126 from monkey sperm, which has been associated with capacitation, reduces the incidence of sperm attachment to epithelium (Tollner et al., 2008, 2012). In men, reduced fertility has been associated with the homozygous inheritance of a sequence variant of the DEFB126 gene (Tollner et al., 2011), indicating that DEFB126 likely plays a role (as yet unknown) in human sperm fertility. Human sperm motility and viability is prolonged by incubation with oviducal epithelium (Kervancioglu et al., 2000); however, a distinct oviducal sperm storage region has not been identified in human oviducts (Williams et al., 1993).
A second possible physical factor is the topography of the oviductal lining. Denissenko et al. (2012) observed that human sperm added to microchannels in vitro tend to remain swimming along walls until they encounter a sharp outward bend in the wall. Then, sperm leave the wall and continue across the microchannel until they encounter another wall. The investigators used their observations to design a circular microchannel with scalloped walls. When human sperm were added to these microchannels, the scalloped pattern acted like a ratchet to force the sperm to travel in one direction around the circle. Whereas the topography of the walls in their microchannel was simpler than the branching mucosal folds lining the human oviduct (Fig. 3B,C), their work indicates that the topography of the oviduct wall could guide sperm migration in vivo.

The third possible physical guidance factor is a thermal gradient along the oviduct. The flagellar beating pattern of human sperm has been shown to change in response to a steep and rapid change in temperature from 31°C to 37°C (and the reverse) (Boryshpolets et al., 2015). When a straw filled with a homogeneous distribution of capacitated human sperm was placed in a thermal gradient of 36.8–42.3°C along 3.5 cm for 20 min, the distribution of sperm became skewed toward the warmer end of the straw (Bahat et al., 2012). A thermal gradient has not yet been detected in human oviducts; however, when the temperatures of the ampullas and lower isthmuses of the oviducts of estrous rabbits were measured surgically, the ampullar lumen was an average of 1.6°C higher than the lower isthmus after ovulation (Bahat et al., 2005). Confirmation of thermotaxis in humans would require detection of a thermal gradient in the oviduct in the periovulatory period in vivo, then testing the response of human sperm in the same gradient in a microfluidic device in the presence and absence of a controlled physiological fluid flow.

Molecular factors that could guide sperm toward oocytes include chemotaxis and/or differential ligand/receptor interactions of sperm with the oviductal epithelium along the length of the oviduct.

There is some evidence for chemotactic guidance of human sperm in the oviduct, particularly toward the cumulus and/or oocyte within the oviductal ampulla. Because of fluid movement caused by ciliary beating, muscle contractions and secretion, and because of the branching mucosal folds that fill the oviductal lumen (Fig. 3B,C), it is thought that chemotaxis can only work over short distances in the oviduct (Suarez, 2015), on the order of millimeters (Perez-Cerezales et al., 2015). Currently, the best candidate for a chemoattractant of human sperm is progesterone. Progesterone is secreted by the cumulus cells that enter the oviduct with the oocyte. In vitro, an orientation response of capacitated human sperm to certain gradients of progesterone has been reported (Oren-Benayo et al., 2008). More recently, capacitated human sperm loaded into a diffusion-based gradient of progesterone formed in a microfluidic device has been reported to travel greater distances into the gradient than controls (Zhang et al., 2015).

Lastly, differential ligand/receptor interactions between sperm and oviductal epithelium along the oviduct could play a role in moving sperm in a pro-ovarian direction. In cattle and mice, sperm will bind to ampullar epithelial cells as well as to isthmic cells (Lefebvre et al., 1995; Chang and Suarez, 2012). Mouse sperm that have reached the ampulla just below the descending cumulus mass were seen to bind tightly to the epithelial surface (Chang and Suarez, 2012). Nevertheless, ligand/receptor interactors have not yet been identified in human sperm and epithelium.

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**Figure 3** Diagrams of cross sections of the human oviduct, illustrating the increasing complexity of the oviductal lumen (black areas) from the uterotubal junction (A), to the isthmus (B) to the ampulla (C). Reprinted from Suarez (2015).

Fortunately, current microfluidics technology offers the opportunity to learn more about how human sperm interact with oviductal epithelium. This could be accomplished by culturing oviductal epithelium in microfluidic channels patterned after the oviductal lumen, then adding sperm to the channels and examining sperm epithelial interactions in the presence of fluid flow. This type of approach has been used, for example, to study how tumor cells enter blood vessels by using microfluidic devices containing microchannels lined with cultured endothelium (Zervantonakis et al., 2012; Wu and Swartz, 2014), and to study responses of human airway epithelium to pollen by culturing airway epithelial cells in microchannels (Blume et al., 2015).

As mentioned above, release of sperm from the oviductal reservoir is gradual. Currently, the mechanism of gradual release of sperm is not known. Nevertheless, once individual sperm are released from the storage reservoir, both physical and molecular factors may guide their subsequent movement toward oocytes in the upper oviduct, which seems to be quite efficient in mice (Muro et al., 2016). One possible physical factor is the pro-uterine current produced by cilia in the human oviduct, which would tend to orient sperm upstream, toward the oocytes (Gaddum-Rosse et al., 1973; Miki and Clapham, 2013).
Microfluidic models for sperm movement, rheotaxis, and chemotaxis

Microfluidic models have emerged to mimic various aspects of the sperm microenvironment, including chemical gradients, surface morphologies and fluid flows, and have helped to reveal a number of biological insights that are difficult to obtain using traditional assays. The key feature of a microfluidic device is the small scale of its topography, from a few to hundreds of micrometers (Xia and Whitesides, 1998; Squires and Quake, 2005). Small channels containing moving fluid make up most of the conduits in a human body. Microfluidic devices provide a physiological platform to recreate the channels and fluid flows in a living system. In addition, the precision of the dimensions of the small structures enables the ability to control the biophysical and biochemical environment at a quantitative level, and at the same time, the results of the experiments can be visualized using optical microscopy. As such, this technology has been employed extensively to study how motile cells and microbes detect and respond to the microenvironment (Kalinin et al., 2009; Kim and Wu, 2012; Wu and Swartz, 2014). Here, we will focus on the use of microfluidic devices to study sperm migration patterns in controlled microenvironments. For studies of ART, we refer to the paper by Gary Smith in this special issue of Molecular Human Reproduction.

Construction of microfluidic devices

Photolithography is a technique that was first developed in the silicon industry to miniaturize electronic components such as transistors. About 20 years ago, this technique was adopted by biologists and chemists for use in making molds for microfluidic devices (Qin et al., 2010). Photolithography is used to make a silicon master with all of the features (channels, inlets and outlets) etched into its surface in a negative pattern. A common process that is used for making a silicon master is to coat a silicon wafer with a thin layer of light sensitive material (photoresist), place a mask of a pattern over the photoresist, and shine light through the mask. In this way, the silicon wafer will be etched into the pattern of interest and can be used as a mold to make multiple microfluidic devices.

The microfluidic devices are made using soft, transparent and non-toxic materials, such as the silicon polymer poly(dimethyl silicone) (PDMS). PDMS can be applied in liquid form to the silicon master and then cured (solidified) by chemical or by UV crosslinking. PDMS has good chemical stability, it is not hydrosopic, and it allows gasses to diffuse to and from the media in the channels, making it an excellent material for living cells (Xia and Whitesides, 1998). The PDMS device is mounted onto a support of PDMS-coated glass. The surfaces of the channels in the device are very smooth and sperm do not stick to the PDMS.

With the recent development of three-dimensional (3D) printing, it is beginning to become feasible to use 3D printers to make microfluidic devices (Ho et al., 2015). Printed devices are highly suitable for prototyping, since they can be made much more quickly than employing the multistep process of photolithography to make a master for molding PDMS devices. Nevertheless, at this time, it is not yet feasible to print with the resolution of 10 μm. Furthermore, PDMS cannot be used for printing microfluidic devices and no better polymers have been developed (Ho et al., 2015). If the resolution, materials and costs of 3D printing eventually meet the needs of biomedical science, not only will use of devices increase rapidly, but also the technology will provide the ability to create devices that can have complex features in the Z-axis as well as the X- and Y-axes (that is, truly 3D microfluidic devices).

Sperm motility and low Reynolds number flow

The patterns of fluid flow around swimming sperm are very different from those of large swimmers such as fish or marine mammals. The small size of the sperm results in a low Reynolds number fluid flow around the sperm. The Reynolds number is a measure of inertial force versus viscous force; it is defined as UL/μ, in which U is the swimming velocity, L is the length of the swimmer and μ is viscosity. For a typical mammalian sperm, the Reynolds number is about 0.01. This allows us to ignore the inertial force when we study the fluid flow around swimming sperm, which leads to a number of interesting behaviors that one does not normally see in large animal swimmers. For example, a number of studies have revealed that sperm tend to swim near a surface. When sperm swim close to a surface, the rotation of the sperm body leads to viscous forces around the body. In this case, the viscous force on top of the sperm head is smaller than the force close to the surface. This non-zero net force leads to a torque that guides the sperm to swim in a circle on the surface (Lauga et al., 2006). Small openings, for example the entrance to the UT, provide large surface areas for the sperm to swim on and so sperm tend to gather at small openings (Tung et al., 2014, 2015a, b).

Most media used for studying sperm motility and IVF are essentially Newtonian fluids. The term ‘Newtonian’ describes a class of fluids where the viscous stress is linearly proportional to the local strain rate. Another way to say this is that, if one has a small sphere settling in a fluid, the viscous force experienced by the sphere is proportional to the settling velocity of the sphere. Fluids without this property are non-Newtonian. Many biological fluids, especially those that contain mucus, are non-Newtonian. The cervical mucus exhibits the non-Newtonian behaviors of shear thinning (viscosity decreases as the strain rate increases) and viscoelastic behavior (there is a component of the deformation that returns to its origin when released from the strain). These fluid properties of cervical mucus affect the pattern of sperm flagellar bending (Suarez et al., 1991).

Sperm swim via undulating the flagellum in a waveform in three dimensions or within one plane. In a Newtonian medium, such as in a typical sperm capacitation medium, a human sperm can swim up to a few hundred μm/s (Nosrati et al., 2015). This makes it challenging to follow the cell dynamics in real time using a compound light microscope. Microfluidic devices can be used to confine sperm movement within a field of view to facilitate live cell imaging (Lopez-Garcia et al., 2008; Frimat et al., 2014; de Wagenaar et al., 2015). Notably, a recent sperm motility study using a microfluidic device revealed that undulating sperm swim within 1 μm of surfaces (Nosrati et al., 2015). Such peculiar swimming behavior is presumably caused by the hydrodynamic interaction between the swimming sperm and the surface.

Sperm rheotaxis

Rheotaxis is a process whereby an organism swims against a flow. Rheotaxis is known in aquatic swimmers such as zebras and copepods (Shang et al., 2008). It has been discovered only recently that
rheotaxis plays an important role in sperm migration (Miki and Clapham, 2013; El-Sherry et al., 2014; Tung et al., 2014, 2015a; Bukatin et al., 2015). Microfluidics provides a convenient way to control flow for sperm. Using various species of mammalian sperm, our lab and others have found that sperm swim against flows through hydrodynamic interactions. More specifically, we found that the back asymmetry of the sperm body facilitates a torque in the presence of the flow, which orients the sperm in the opposite direction of the flow. Rheotaxis of sperm is robust, and it exists in both Newtonian and non-Newtonian fluids (Miki and Clapham, 2013; Tung et al., 2015a; Bukatin et al., 2015).

**Sperm chemotaxis**

Chemotaxis is a process whereby cells or organisms move up a gradient of a chemical attractant. Microfluidic devices give us the ability to create well-defined gradients and allow for quantitative analysis of sperm movement in the gradients (Xie et al., 2010; Chang et al., 2013; Zhang et al., 2015). A number of chemotaxis chips have been developed that are suitable for sperm chemotaxis investigations. In our labs, we have developed a hydrogel based microfluidic device, which has been used successfully for sperm chemotaxis studies (Chang et al., 2013). In this device, three parallel channels (Fig. 4B) are patterned into a 1 mm thick agarose gel membrane, which is sandwiched between a Plexiglass manifold and a stainless steel support frame (Fig. 4A). Chemoattractant and buffer are introduced along the two side channels, while sperm are introduced into the center channel. Chemoattractant diffuses through the agarose gel wall, and establishes a linear chemical gradient across the center channel. Here, as long as the concentration of chemoattractant in the one side channel and the flows through both side channels are kept fixed, the chemical gradients can be kept at a constant value for a long time; in our labs, we are able to maintain the chemical gradient for a few days (Kim et al., 2010; Kim and Wu, 2012).

**Microfluidic sperm sorter**

Knowledge learned about sperm swimming has started to allow us to design microfluidic devices that manipulate sperm according to their swimming behavior. One example is to design microfluidic sperm sorters to separate swimmers of different speed. Chung et al. (2006) separated fast swimming sperm from immotile sperm based on their differential diffusion coefficient. Two streams, one with sperm and one without, meet in a T-junction, and flow together into one channel. Because motile sperm have higher diffusion rates than the immotile sperm, this leads to motile cells crossing over into the empty stream and being selected for further analysis. As we learn more about sperm swimming behavior, we anticipate a new generation of sperm sorters, with the potential for use in ART, using microfluidic devices.

**Conclusions**

In summary, we have described a number of factors that could be responsible for guiding human sperm through the female tract to oocytes. Redundancy of guidance factors in various segments of the tract could provide greater assurance that appropriate numbers of capacitated sperm will reach the oocytes shortly after ovulation, so that fertilization can proceed quickly and with low incidence of poly-spermy. Possible cooperation among factors remains to be studied. Microfluidics offers new opportunities to improve our understanding of human sperm migration and to use this understanding to prepare sperm for intruterine insemination, IVF and ICSI.

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