Disrupting the wall accumulation of human sperm cells by artificial corrugation

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Many self-propelled microorganisms are attracted to surfaces. This makes their dynamics in restricted geometries very different from that observed in the bulk. Swimming along walls is beneficial for directing and sorting cells, but may be detrimental if homogeneous populations are desired, such as in counting microchambers. In this work, we characterize the motion of human sperm cells ~60µm long, strongly confined to ~20µm shallow chambers. We investigate the nature of the cell trajectories between the confining surfaces and their accumulation near the borders. Observed cell trajectories are composed of a succession of quasi-circular and quasi-linear segments. This suggests that the cells follow a path of intermittent trappings near the top and bottom surfaces separated by stretches of quasi-free motion in between the two surfaces. We show that the introduction of artificial petal-shaped corrugation in the lateral boundaries limits the accumulation near the borders and contributes to increase the concentration in the chamber interior. The steady state limit is achieved over times of the order of minutes, which agrees well with a theoretical estimate based on the assumption that the cell mean-square displacement is largely due to the quasi-linear segments. Pure quasi-circular trajectories would require several hours to stabilize. Our predictions also indicate that stabilization proceeds 2.5 times faster in the micro-corrugated chambers than in the non-corrugated ones, which is another practical reason to prefer the former for microfluidic applications in biomedicine.

I. INTRODUCTION

Motility is a crucial reference parameter in fertilization studies since it is an unequivocal indicator of sperm viability. Under natural conditions a motile spermatozoon is needed for successful oocyte fertilization. When normal fertilization repeatedly fails, the infertile couple may resort to assisted reproduction techniques, where the gametes are isolated and put together to induce in vitro fertilization. Nowadays, sophisticated sperm cell preparation techniques, most of which require motile cells, are available to retrieve the best physiological sperm for assisted reproduction1–4. For instance, higher DNA integrity in the sperm selection has been recently achieved with innovative microfluidic devices5. The World Health Organization classifies semen quality based on sperm motility (asthenozoospermia) and the number of sperm cells (azoospermia), among others parameters6. Thus, a sufficient number of motile cells and their precise identification are important for the clinical diagnosis associated to male infertility.

All of the microfluidic devices employed to evaluate sperm motility or sperm number4,5,7–14 (e.g. a drop of solution confined between two glasses or inside a microfluidic device) present one or more boundaries. The attractive interaction of sperm cells with these boundaries increases their dwell time in their neighborhood15–17 (in the case of extreme 2D confinement all cells should go to the border18). Consequently, errors may be introduced in the evaluation of sperm motility or sperm count. For example, in the standard Makler counting chamber10, widely used today in andrology laboratories to evaluate sperm motility6, the sperm motility count is performed in its central area, whereas a circular border is formed by the medium-air interface containing the cells. According to the natural tendency of the spermatozoon to attach to the borders, spermatozoa move away from the bulk and accumulate near the boundary. In mL containers and considering the typical sperm cell speeds in the range of 30-100µm/s, cell accumulation takes place over short time scales, of the order of a few minutes. As a consequence, the sperm number in the counting area decreases with time during the sample examination. Besides, since progressive motile sperm cells tend to reach the border faster, these cells will be trapped by the borders earlier, leading to an inaccurate diagnosis if evaluated several minutes after loading the chamber. This effect may be of minor importance in typical mL volume chambers, but will certainly become a dominant issue in the miniaturized micro or nanoliter volume chambers used in microfluidic lab-on-chip devices (platforms all-in-one)14,5,20–22. In other words, it would be highly desirable to find a way to counteract the material-independent and ubiquitous cell accumulation at the chamber boundaries with a mechanism leading to the formation of a more uniform density distribution of microswimmers.

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Accumulation of spermatozoa at liquid-wall interfaces is a phenomenon common to many self-propelled microorganisms confined to different surfaces, from glass, PDMS or SU8 to air\cite{23–30} and even to cell membranes\cite{31}. Recently we used this property to direct and concentrate sperm cells through the geometrically induced rectification of the cell motion by asymmetric U-shaped obstacles\cite{32}. This ratchet effect in effectively two-dimensional systems becomes more pronounced for large perimeter-to-surface ratios and therefore lets us envisage the design and manufacture of miniaturized devices to control the cell dynamics as well as the artificial microswimmer dynamics for selection, testing, concentration, separation\cite{33, 34}, or trapping\cite{35, 36}.

In this work we demonstrate that, by properly texturing the sample borders, it is possible to tune the relative density of cells in the interior of a shallow chamber by diminishing the sperm density at its perimeter. In addition, we provide further insights on the motility of human sperm cells confined between two surfaces separated by $\sim 20 \mu m$, a distance shorter than their own length, $\sim 60 \mu m$. In particular, we show that the head oscillation and the VCL (curvilinear velocity) significantly decrease when sperm swim next to the borders. This parameter could eventually be used as a quantitative indicator of the confinement dimensionality of the system. Based on the observation that most microswimmers are attracted to obstacles\cite{17, 23, 24, 27, 31, 32, 37, 38}, we expect that, when a pair of confining parallel surfaces are close (less than a few tens of $\mu m$ apart), a sperm cell, having relatively large size and strength, will not be completely confined by any of the walls. Instead, since the attraction basins compete with each other, we expect the state of each cell to fluctuate between temporary trappings near the top and bottom surfaces and short “runs” in the central regions. A typical cell trajectory will thus be composed of an intermittent succession of quasi-circular arcs and quasi-linear segments. The dynamics of cell accumulation at the borders will be determined by the nature of these trajectories. In this paper we examine this problem in detail.

II. MATERIALS AND METHODS

A. Micro-fabrication of the chambers

Quasi 2D shallow chambers were prepared in SU-8 negative optical resists deposited on glass substrates. These materials ensure (a) optical transparency allowing transmission microscopy imaging, (b) high aspect ratio structures, (c) biocompatibility, (d) watertightness, and (e) high resolution for nano-fabrication. We fabricated two different types of circular chambers, one having a smooth circular perimeter, as in a Petri dish, the other having a micro-structured border with concave semi-circular cavities distributed periodically as in a flower or rosette design (see Fig. 1). The depth of the shallow chambers is $d = 25 \mu m$.

B. Sperm preparation

Human sperm were separated from the seminal plasma by migration sedimentation\cite{19}. The diluted highly motile sperm population was adjusted to $10^7$ cells/ml with BWW medium, supplemented with 1% human albumin. Then, the sperm were kept in an incubator at 37° C with 5% CO$_2$ in air until their use. The chambers were loaded with 1-2 $\mu l$ of sperm suspension over the well and covered by sliding a coverslip. Notice that the inoculation is not localized at the center of the chamber with a micropipette, but almost uniformly distributed. In order to properly seal the chambers, excess liquid was removed and the edges were covered with mineral oil to prevent air entry. The sperm movement was recorded by phase contrast video-microscopy using a digital camera (Nikon, USA) connected to an inverted microscope (see Fig. 1(c)). Recordings of tracks were performed from 7.5 to 30 Hz with a resolution of 1280x1024 pixels with the BR Nis Elements software (Nikon, USA) and the image analysis was made with ImageJ free software (NIH, USA).

III. RESULTS AND DISCUSSIONS

A. Effect of confinement on the motility of sperm cells

By analyzing the morphology of the cell trajectories in the interior of the chambers we are able to identify the z position of the swimmer. Indeed, when looking from above the surface (see sketch of measurements in...
FIG. 2. Human sperm confined motility characterization. (a)-(e) Upper panels: examples of typical observed trajectories. Lower panels: cartoons showing the corresponding cell locations relative to the confining surfaces (out of scale because sperm cells are larger). The proportions of cells of each class are indicated. (f) Measured VCL and VSL for the different trajectory types. 179 cells were considered. (g) Average VCL speeds for 62 cells arriving at the border of the shallow chamber and for 94 cells leaving its perimeter. Rightmost panels: images of cells arriving at the border and leaving it. * indicates significant differences ($p < 0.05$).

Fig.1(c)), pusher cells swimming in the neighborhood of the SU-8 surface will tend to turn clockwise$^{25,37,40}$, as shown in Fig.2(b-c), while cells navigating close to the glass coverslip surface will be observed as following circles in the opposite direction, CCW, as in Fig.2(d). We have also identified trajectory segments where the cells do not swim in circles, which we label linear trajectories, Fig.2(a-b). Cells following these paths may either swim in between the top and bottom surfaces or, more likely, touch one of them changing the direction of the movement and swimming back in between the surfaces. The latter is also the case with about 60% of those trajectories labeled as irregular, Fig.2(e), which change curvature exhibiting a winding path. If the cells move directly from the neighborhood of one surface to the neighborhood of the other, eight-shaped and S-shaped trajectories may be observed (see Fig.2(e) and Movies in the Supplementary Material$^{41}$).

It is instructive to compare this analysis with previous studies in sea urchin sperm where the trajectories, which are helicoidal in the bulk, become circular near a surface$^{40,42}$. Guerrero and coworkers studied the circular swimming behavior of L. pictus and S. purpuratus spermatozoa at a glass-water interface finding an average radius $\rho$ of 24.9 $\mu$m for L. pictus and of 17.8 $\mu$m for S. purpuratus$^{43}$. The swimming patterns of mammalian sperm are more heterogeneous$^{34,45}$. High resolution 3D dynamic tracking of human sperm has shown that in the prevalent swimming pattern the sperm head moves forward swiftly (as fast as 140 $\mu$m/s) along a slightly curved axis with a small lateral displacement$^{46}$. These authors have also shown that 4-5% of motile cells swim along well defined helices, whose radius is approximately 0.5-3$\mu$m and whose linear speed is in the range between 20 and 100$\mu$m/s$^{46}$. About 90% of these helicoidal trajectories are right-handed. The influences of a Poiseuille shear rate and of viscosity on the motion of a mammalian sperm cell near a surface have been recently investigated as well$^{47}$. In summary many recent experiments have shown how the sperm motility near surfaces changes considerably in comparison with its free dynamics. However a complete characterization of human spermatic cells under strong confined motility is important and still lacking. This characterization is crucial for designing optimal new microfluidic devices in view of medical applications$^{48}$. In addition it could be helpful for testing the good performance of the present protocols of semen analysis and the clinical laboratories accreditation, which are using non-propelled latex beads$^{49}$.

In our results of Fig.2 the shallowness of the chambers and the limited resolution of the tracked trajectories do not permit us to investigate in detail the transition between the patterns reported in 3D environments$^{28,42}$ and those of the quasi-2D trajectories reported here. On the other hand, the different chirality (CW or CCW) of the circular paths that we observe does evidences the switch-
ing between top- and bottom-swimming (see the Movie in the Supplementary Material\(^{41}\)). This fact has also been reported for other pusher microswimmers such as \(E. \text{Coli}\) confined in 25\(\mu\)m deep chambers\(^{50}\).

It is also instructive to investigate the speed of sperm cells in the confined environment. Let us first describe the three different approaches typically used in the biomedical community for characterizing the sperm cell velocity: (1) the VCL, which is the curvilinear velocity of the sperm head along its trajectory (including the oscillations). (2) The straight-line velocity (VSL), which is the average velocity along a straight line joining the ends of a given track. (3) The average path velocity (VAP), which is the average velocity of a cell over a smoothed path\(^{31}\).

A quantitative indicator of the degree of confinement can be obtained from the curvilinear velocity VCL. Indeed, cells swimming in a border-free three dimensional open medium acquire an unrestricted (i.e. maximum) swimming velocity VCL whereas cells in proximity to one border should exhibit a reduced VCL due to the cell-wall interaction. This velocity will be further reduced if the cell motion is limited to two dimensions, as it is the case in the chamber interior, where cells are confined between a glass coverslip and the chamber bottom made of SU-8 material. Reports on human sperm cells\(^{16}\) show that unconfined cells swim with a VCL of 88.0 \(\pm\) 28.7 \(\mu\)m/s and a lateral head displacement of 5.4 \(\pm\) 2.9 \(\mu\)m.

In contrast to that we find that in shallow chambers the VCL is roughly independent of the morphology of the cell trajectories as shown in Fig. 2(f), and is substantially smaller than that recorded in the bulk, thus confirming that in all cases the cells remain influenced by the proximity of the surfaces. Indeed, by analyzing 179 cell trajectories in non-corrugated chambers (see Fig. 1) we found that the VCL (mean \(\pm\) S.D.) is 42.6 \(\pm\) 6.1 \(\mu\)m/s in the interior of the chambers, i.e. smaller than the value reported for unconfined swimming\(^{16}\). Furthermore, for cells swimming next to the borders, the VCL decreases from the interior value to 34.2 \(\pm\) 9.3 \(\mu\)m/s, which represents a substantial reduction (Fig. 2(g)). When the cells leave the border, the VCL changes from 36.0 \(\pm\) 7.0 \(\mu\)m/s along it to the interior value (Fig. 2(g)), thus indicating that when a cell leaves the border it keeps no memory about its interaction with the wall. The VSL parameter, straight-line velocity, indicates how far a cell has traveled in a certain unit time. As expected, the linear trajectories are characterized by a larger VSL. We have also corroborated that cells swimming near the borders have approximately the same probability of heading right and left. A more detailed analysis of the cells following nearly circular trajectories is shown in Fig. 3, where we present the relative distribution of their curvature radii irrespective of the chirality of the trajectory. This distribution exhibits a maximum at about 70 \(\mu\)m, a long tail of high values, and is well approximated by

\[
\gamma(k, \theta; \rho) = \rho^{-k} \exp(-\rho/k)/\theta^k \Gamma(\theta),
\]

with \(k = 0.044\) and \(\theta = 3.970\). Here \(\Gamma(k)\) is the usual gamma function. Due to rotational diffusion, the cells never follow precisely a circular path. Those approximately following circles will diffuse very slowly, as we will show in Sec.3.3.

We remark that the mean radius of these circles is considerably larger than that found for other sperm species under a 2D confinement\(^{15}\).

B. Effect of confinement on the distribution of cells

Whereas the top and bottom surfaces confining the cells' motion determine the morphology of the trajectories, the borders induce a highly inhomogeneous population of cells, which tend to accumulate next to the perimeter of the chamber. Indeed, as we observed before for rectangular chambers\(^{12}\), spermatozoa accumulate near the border and their density rapidly diminishes within a distance of 25 \(\mu\)m to reach a nearly constant value in the chamber interior. In order to reduce the inhomogeneity induced by the borders we microstructure them in such a way as to force the re-injection of cells attached to the perimetric walls into the interior of the chamber. A similar study has been done for artificial (dry) self-propelled objects of macroscopic size under shaking and in the high density limit\(^{52,53}\). When measuring the cell density at the border as a function of time, we observe that for the non-corrugated chambers, this density increases in time and reaches a value between two and three times larger than in the center of the chamber after 5 minutes of equilibration, Fig.4(b). In contrast to that, for the rosette design, the density at the border is roughly constant over the observation time, Fig.4(c), and very similar to the value in the interior of the chamber during all the experiment (see the density ratio in Fig.4(d)). In the rosette experiment, the equilibration time is apparently shorter than the two minutes elapsed between the inoculation and the first image.

For the rosette geometry, it is important to analyze the distribution of cell departure angles when leaving a petal. Indeed, this angle gives us information about the relative positions of the flagellum and the surface. Some
FIG. 4. Sperm cell density in shallow chambers. (a) Location of the typical domains used to count the cells in the chamber border and interior. (b) Evolution of the cell concentration near the border and in the interior of a non-corrugated chamber. (c) Evolution of the cell concentration near the perimeter and in the interior of a rosette-shaped chamber. (d) Cell density ratio, comparing border vs interior accumulation. In all cases shown, the chamber radius is \( R = 3.5 \text{ mm} \).

representative trajectories are shown in Fig.5. The mean departure angle, measured as indicated in Fig. 5, is \( \langle \alpha \rangle = 74.9^\circ \pm 17.7^\circ \). This is close to the values reported by Denissenko et al., who measured the angle of human sperm departure from a 90° bend of a microchannel. It is also consistent with the results of Kantsler et al. for bull spermatozoa scattered upon arrival at a corner. Another important parameter useful to characterize the sperm motility close to a border is its head oscillation amplitude, a parameter of their head center movement along the trajectories. Here it is worth to emphasize that, as can be observed in Fig.5(a), all head oscillations are strongly influenced by the boundaries, reducing considerably their amplitude (as has been previously reported in shallow rectangular microchambers, see the supplementary material in Guidobaldi et al.\(^{32}\)). In addition we have observed that the typical head oscillation amplitude is recovered quickly after leaving the borders, within a few microns. This observation reinforces the idea that no memory of the wall influence is observed in the motility in the interior of the chamber. This is a key observation for the formulation of theories and numerical simulations of confined swimming.

FIG. 5. (a) Exit angle for cells leaving a petal of the rosette. Representative trajectories. The mean angle over 48 observations is \( \langle \alpha \rangle = 74.9^\circ \pm 17.7^\circ \). Note the marked compression of the head oscillations when the cell swims next to the borders, surrounded on three sides. (b) Exit angle frequency distribution.

C. Theoretical estimates

When smooth-border chambers are used, experiments show initially an increase in the cell population near the border, which after some time saturates. The saturation time can be estimated by assuming that cells undergo normal diffusion inside a circular chamber of radius \( R \), and that they are absorbed when they reach the boundary. In this case, we must solve the diffusion equation,

\[
\frac{\partial n(r,t)}{\partial t} = \frac{1}{r} \frac{\partial}{\partial r} \left[ r D \frac{\partial n(r,t)}{\partial r} \right],
\]

where \( n \) is the cell density in the chamber interior subject to the boundary condition \( n(R,t) = 0 \) and the initial condition \( n(r,0) = n_0(r) \), and \( D \) is the diffusion coefficient. The solution to Eq. (1) can be expressed as a series of the form

\[
n(r,t) = \sum_{n=1}^{\infty} F_n(r) \exp(-D\alpha_n^2 t).\]

where the functions \( F_n(r) \) are inner products of Bessel functions and the initial condition in the circular domain, and the \( \alpha_n \)'s are the roots of the Bessel function \( J_0 \), \( J_0(R\alpha) = 0 \). Since \( Ra_1 = 2.40 \) and \( Ra_2 = 5.52 \), at long times we may keep only the first term in the series, which allows us to define a sample equilibration time,

\[
\tau_S = \frac{1}{D\alpha_1^2}.
\]
It is easy to see that Eq. (3) is a good long-time approximation for almost any $n_0(r)$.

If rosette geometries are used instead, a different approach is needed to estimate the equilibration time. Indeed, in this case the sperm cells reside on the wall an average time $T_R = L/2v_B$ before returning to the sample interior. Here $L$ is the length of the petal and $v_B$ the speed along the border. For $L = 1$ mm and $v_B = 34$ $\mu$m/s, $T_R \approx 15s$, an interval that is short compared with the experimental times. Therefore, for these chambers, it is a reasonable approximation to use reflecting boundary conditions at $r = R$. This leads to a result similar to Eq. (3), except that we must replace the zeroes of $J_0(R \alpha)$ by those of $J_1(R \beta)$ (again, this is valid for almost any initial condition). This leads to a new equilibration time,

$$
\tau_R = \frac{1}{D \beta_1^2} \quad (4)
$$

Since $\beta_1 R = 3.83$, we readily find that $\tau_S = 2.53 \tau_R$, i.e., the rosette geometry leads to faster sample equilibration.

The diffusion coefficient depends on the type of motion considered. For quasi-circular motion with angular frequency $\Omega$, Schimansky-Geier et al.\textsuperscript{57} showed that,

$$
D_1 = \frac{D_r v^2}{2(D_r^2 + \Omega^2)} \quad (5)
$$

where $D_r$ is the rotational diffusion coefficient and $v$ is the linear speed\textsuperscript{57,58}. If the mean square displacement is determined by the motion in quasi-linear intervals, the diffusion coefficient is given by,

$$
D_2 = \frac{v^2 T}{2(1 + D_r T)} \quad (6)
$$

where $T$ is the duration of the quasi linear steps\textsuperscript{59}. Large values of $T$ increase the mean square displacement, but this effect is weakened by rotational diffusion, which is more effective for longer paths.

The equilibration times $\tau_R$ and $\tau_S$ can be estimated using observational values of the parameters. In the case of the smooth-edged chamber, if we take $\Omega = 0.6s^{-1}$ (corresponding to the most frequent radius, see Fig. 3), $D_r = 0.01rad^2/s^{\cdot2}$, and $v = 38\mu$m/s (estimated VAP far from the border), we obtain for quasi-circular trajectories $D_1 \approx 20(\mu$m$^2$/s) and $\tau_{S1} = 0.0086(s/(\mu$m$^2$/s)$^2)$, which, for $R = 3.5$mm yields $\tau_{S1} \approx 29$hrs. This is much larger than the experimental times.

On the other hand, for quasi-linear trajectories we find $\tau_{S2} = 0.00024(s/(\mu$m$^2$/s)$^2$)$0.01s^{-1} + T^{-1}$) $R^2$. Due to the irregularity of the trajectories it is difficult to determine precisely an average “run” time, but observation of the experimental tracks indicates that a typical length is of about 400 $\mu$m, which would correspond to $T \approx 10.5$s.

Although this parameter is the one that can be determined with the less precision, $\tau_{S2}$ is not very sensitive to changes in $T$. For all parameter values of interest, $\tau_{S2} < \tau_{S1}$ and equilibration is essentially determined by motion in the quasi-linear regime. The same arguments hold for the rosette-shaped chambers.

If $R = 3.5$mm and $T = 10.5$s, $D_2 \approx 6900(\mu$m$^2$/s) and $\tau_{S2} \approx 300s$. However, since at any given time, only about one third of the cells are either in the quasi-linear or the winding state (irregular path), the stabilization time $\tau_S \approx 3\tau_2 \approx 900$s. Since the boundary does not absorb all cells, this time must be taken as an upper bound for the period required to establish the steady state. The results reported in Fig. 4(b) indicate a stabilization time of about 5 minutes, which is compatible with this bound. A sample from a different donor (not shown here) had a stabilization time closer to 15 minutes.

For the rosette-shaped chambers, we get $\tau_R \approx 350s$. Why is the equilibration faster than this in the experiment shown in Fig. 4? A possible explanation is that the procedure to fill the chamber creates an initial condition in which the sperm is almost uniformly distributed. If this is the case, the reflecting boundary conditions and the low capacity of the borders to absorb sperm cells warrant that the initial state is already very close to equilibrium. Therefore the sample evolution is not observed in the experimentally accessible time scale.

### IV. SUMMARY AND CONCLUSIONS

The physics of microswimming\textsuperscript{60} in bounded and unbounded media has become the focus of an intense research effort. Sperm cells are pushed by the whip-like oscillation of their flagella and interact in a characteristic fashion with confining surfaces or obstacles\textsuperscript{17,25,28,32,45,55}. On the other hand, flat cell counting chambers may become integral parts of miniaturized lab-on-a-chip devices designed to manipulate sperm. For this reason, our aim is to characterize human sperm dynamics in quasi 2D extremely shallow containers, using non-corrugated and corrugated-chambers. In our experiments the chamber bottom and top are separated by a 25 $\mu$m gap, a smaller distance that the sperm cells length. Our main findings are as follows:

- Cell trajectories are a succession of quasi-circular and quasi-linear segments. This suggests that the cells follow a path composed of temporary trappings near the SU-8 surface (CW arcs) and near the glass coverslip (CCW arcs), separated by stretches of quasi-free motion in the intervening gap. The radial frequency of the quasi-circular paths close to the surfaces has also been analyzed, giving a widely spread population with a mean value of $\sim 70\mu$m. The large distribution width express the heterogeneous dynamics of the cell population.

The typical path curvature radius in our chambers,
Fig. 3, is much larger than the 0.5-3 \( \mu \text{m} \) observed in 3D helicoidal swimming trajectories when viewed “end-on”\(^6\). A similar, albeit less drastic, enlargement of the curvature radius when going from 3D to 2D was previously seen in sea urchin sperm. The radius for the two-dimensional orbits for sea urchin sperm is smaller\(^4\) than that for human sperm. This is likely to be due to the higher strength of the propelling force for the former. Note that the propelling force is proportional to the cell speed in the bulk, which is about twice as large for a sea urchin as for human sperm\(^2\),\(^3\),\(^4\).

• Cells accumulate near the curved border, as it had been previously shown they do near rectilinear borders. An original use of micro-structured petal-shaped border limits accumulation near the perimeter and would contribute to increase the concentration in the interior of a miniaturized chamber. This may be compared to the use of concave indentations that trap sperm near the boundary\(^3\).

• Cell motion is slower in the shallow chambers (2D) than in the bulk (3D). An important speed reduction had already been measured in sea urchin sperm\(^2\). In addition we show for the first time that cells further slow down by 27% when they move parallel to the curved borders. This observation was not unexpected since close to the borders the confinement is stronger due to the hydrodynamic attraction to the three surfaces involved.

• The amplitude of the head oscillation along borders is clearly reduced. This is evidence for a drastic change in motility under strong confinement. The exit angle for cells leaving the petals has been measured and a quick recovery of the head amplitude oscillation was observed at a few microns from the borders.

• The dynamics of cell accumulation near the borders was studied and it was found that several minutes are needed to reach a steady state in non-corrugated chambers having diameters of the order of the millimeter. This agrees with a theoretical estimate that assumes that the cell mean-square displacement is largely due to the quasi-linear trajectory segments. Quasi-circular trajectories, on the other hand, would require several hours to stabilize. Our theoretical estimates also show that stabilization proceeds 2.5 times faster in the rosette geometries than in the smooth chambers, which is a second practical reason to prefer the former for biomedical applications\(^4\).

Taken together with previous findings\(^3\),\(^2\), our results underscore the nontrivial effects that the thickness of the confining chamber, the shape of an obstacle and the texture of the borders have on the dynamics of human sperm cells. These new insights on the extremely confined sperm dynamics should be included in phenomenological models used to optimize the design of sperm microfluidic concentrators and sorters\(^4\),\(^3\).

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