Cellular geometry controls the efficiency of motile sperm aggregates

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Sperm that swim collectively to the fertilization site have been observed across several vertebrate and invertebrate species, with groups ranging in size from sperm pairs to massive aggregates containing hundreds of cells. Although the molecular mechanisms that regulate sperm–sperm adhesion are still unclear, aggregation can enhance sperm motility and thus offer a fertilization advantage. Here, we report a thorough computational investigation on the role of cellular geometry in the performance of sperm aggregates. The sperm head is modelled as a persistent random walker characterized by a non-trivial three-dimensional shape and equipped with an adhesive region where cell–cell binding occurs. By considering both, a simple parametric head shape and a computer reconstruction of a real head shape based on morphometric data, we demonstrate that the geometry of the head and the structure of the adhesive region crucially affects both the stability and motility of the aggregates. Our analysis further suggests that the apical hook commonly found in the sperm of muroid rodents might serve to shield portions of the adhesive region and promote efficient alignment of the velocities of the interacting cells.

1. Introduction

In most species, the number of sperm available for fertilization far outnumber ova, which gives rise to fierce competition at the cellular scale [1]. The highly efficient and streamlined shape of most sperm cells is the product of intense selective pressure to improve swimming performance. In a rare number of systems, sperm form cooperative groups, or aggregates [2–6]; this fascinating behaviour is believed to improve the swimming performance of the cells involved, compared to individual cells, and therefore the chances of successful fertilization [6]. Yet, in vitro studies have shown inconsistent results [3] and the underlying physics of the associations remains elusive [7–9].

Among mammals, the natural variation observed within muroid rodents [10] offers important insight into how cell shape and orientation can mediate collective motion of sperm. For example, the sperm of house mice (Mus musculus) form groups in which the head of a sperm is bound to either the head or the tail of another cell, whereas in the Norway rat (Rattus norvegicus), sperm form comet-like aggregates in which all the cells are bound at the head and preserve the head-tail directionality of a single cell [11]. These morphological variations result in a substantial difference in the aggregate swimming performance: while in the Norway rat, sperm groups swim faster than single cells, no speed advantage is found in the house mouse. Train-like aggregates are also found in the wood mouse (Apodemus sylvaticus), where aggregation is known to improve swimming performance [2]. In most muroid rodents, sperm have a falciform head with an apical hook that is thought to facilitate the formation [2] and/or stabilization [11] of aggregates, although other scenarios have also been suggested [12]. Although the molecular mechanisms that regulate sperm–sperm adhesion in these systems are not well understood, in house mice,
transmembrane glycoproteins more typically associated with sperm-egg binding are probably involved [13].

In previous work, we studied sperm from *Peromyscus* rodents and analysed how the swimming velocity of the aggregates is modulated by group size [14]. By combining fine-scale imaging of living cells and a simple two-dimensional mechanical model of sperm aggregates (inspired by the Vicsek model [15] and the statistical mechanics approach to collective behaviour [16–24]), we demonstrated that the average velocity of an aggregate does indeed increase with its size as the group moves more persistently. This benefit, however, is offset in larger aggregates as the geometry of the group forces sperm to swim against one another. This result is a non-monotonic relationship between aggregate size and average velocity with an optimum that is predominantly determined by the geometry of the sperm head [14]. The underlying mechanism leading to this optimality is straightforward: by forming tightly packed head-to-head aggregates, sperm are able to ease each other’s directional fluctuations by sterically restraining one another. This results in a straighter trajectory, thus a greater average velocity. Large aggregates, however, tend to be isotropic and this forces the sperm to swim against one another, thus reducing the aggregate speed.

In this work, we report a thorough computational investigation on the role of cellular geometry in the performance of sperm aggregates, as well as on the structure of the adhesive region of the cell head. For this purpose, the sperm head is modelled as an ellipsoid of revolution. Although considerably less structured than the actual sperm head is modelled as an ellipsoid of revolution. (e.g. slenderness, oblateness, etc.) and provides insight into the effect of the head shape on the spatial organization of sperm aggregates, as well as on the structure of the adhesive region, and identify a number of highly efficient configurations that could provide a basis for future experimental studies.

2. The model

Our computational model aims to reproduce the physical interactions between sperm cells arising from steric repulsion and linkage through adhesive molecules. Each cell is modelled as a persistent random walker in three dimensions (e.g. [25]), subject to a constant propulsive force and a random torque, and whose dynamics is governed by the overdamped Newton equations. When two sperm cells come into contact they experience a repulsive force, sufficient to prevent them from overlapping, as well as an attractive force reproducing the effect of adhesive molecules. The sperm head is modelled as a three-dimensional simplex: a set of triangles connected along the edges in such a way to form a closed polyhedron. This allows us to reproduce any desired morphological features including the apical hook found in the sperm cells of most muroid rodents [10]. The simplest non-trivial three-dimensional shape suitable for a geometrical model of the sperm head is the ellipsoid of revolution (figure 1a). Although less complex than the typical head shape found in rodents, this is a simple and yet sufficiently parametric shape, whose morphological feature can be entirely described in terms of the three parameters $a$, $b$ and $c$ (figure 1a). Adjusting the ratio between these lengths permits us to reproduce various kinds of shapes (figure 1b).

At each time-step $t$, the location and orientation of the head of the generic $i$th sperm cell are described by the position $r_i(t)$ of the head centre of mass and the direction $p_i(t) = R_i(t) \cdot p_i(0)$ of the head major axes, with $R_i(t)$ a $3 \times 3$ rotation matrix and $p_i(0)$ the direction at $t = 0$. These quantities are then iteratively updated by means of the following recursive equations:

\[
\begin{align*}
    r_i(t + \Delta t) &= r_i(t) + \Delta t[v_0 p_i(t) + \mu_i F_i(t)] \quad (2.1a) \\
    R_i(t + \Delta t) &= R_i(t) \cdot T_i(t). \quad (2.1b)
\end{align*}
\]

The term $v_0 p_i$ in equation (2.1a) represents the self-propulsion provided by the beating of the tail, $\mu_i$ is a mobility constant and $F_i$ expresses the total force experienced by the $i$th head. In turn, this can be decomposed as:

\[
F_i = \sum_{j=1}^{N_i} F_{ij}^{\parallel} + \sum_{j=1}^{N_i} F_{ij}^{c},
\]

where $F_{ij}^{\parallel}$ and $F_{ij}^{c}$ are, respectively, the forces resulting from the steric and adhesive interactions between the $i$th sperm head and other heads, we have dropped time-dependence for sake of conciseness. $N_i$ is the number of cells in contact with the $i$th sperm head, while $N_s$ is the number of cells connected to the $j$th head by an adhesion molecule. Finally, $T_i$ is a $3 \times 3$ matrix, that embodies the rotation caused by torque experienced by the $i$th sperm head.

Steric interactions are resolved by locating the intersections between the triangles comprising the three-dimensional model of the head. This task is performed using the C++ library RAPID [26]. Once a collision is detected, we reconstruct the volume defined by the intersection between the overlapping heads. The position $r_{ij}^s$ of the centre of mass of such a volume is identified as the location of the collision between the $i$th and $j$th cell (figure 2a). The associated steric force $F_{ij}^{\parallel}$ is parallel to the spatially averaged surface normal, $N_{ij}$, of the intersecting regions, while its magnitude is set to be...
and interaction with the and torque on the and connecting them with a Hookean spring. The spring has finite strength and will break if  \( r_{ij} \leq l_0 \) for adhesion or not. The simulations are initialized by placing the same mesh, with triangles being designated either adhesive or not. The simulations are initialized by placing the cells at random positions with random orientations within a small region. Overlaps between the cells are removed and the density is increased to form an aggregate. The simulations then undergo 10^5 equilibration steps to obtain a stable configuration before starting the measurements. We assume ergodic behaviour in our simulations and unless stated otherwise, results presented are based on a single simulation of at least \( t = 10^7 \) time steps.

3. Results

3.1. Aggregates travel faster than individual cells

Each individual in silico sperm swims forward while undergoing rotational diffusion, mimicking the erratic swimming behaviour of real sperm. There is no preferred direction or target in the simulation, therefore sperm cells perform a persistent random walk (figure 3a). This motion is characterized by a short-time ballistic regime, in which the mean square displacement of the centre of mass of the aggregate scales quadratically with time (i.e. \( \langle R^2(t) \rangle \sim t^2 \)), where the angular brackets indicate a statistical average), and a diffusive regime, in which \( \langle R^2(t) \rangle \sim t \). The ballistic motion occurs over a time interval that is shorter than or comparable to the reorientation time of the sperm. The diffusive regime, on the other hand, is found when the time interval is greater than the reorientation time of the sperm (figure 3b).
When many cells crowd together, the available space for each sperm head is restricted, leading to a reduction in the amount of rotational motion each sperm head experiences while swimming (figure 3a). This results in a longer reorientation time for a sperm aggregate, thus a larger persistence (figure 3b). As a consequence, the distance covered by a sperm aggregate within a given time interval is larger than in the case of individual cells, effectively making aggregates faster. Such a benefit, however, also depends on the travelling time. For instance, as shown in figure 3b, groups of $N = 4$ sperm cells are faster than groups of $N = 16$ only in short time periods, whereas this pattern reverses at longer durations.

Whereas the average velocity (i.e. $V(t) = \sqrt{\langle R^2(t) \rangle}/t$ for finite $t$) increases with the size $N$ of the aggregates, their speed (i.e. limit of $V(t)$ for $t \to 0$) is a monotonically decreasing function of $N$. This originates from the fact that, owing to their ellipsoidal shape, cells are always partially splayed towards the mean direction of motion of the aggregate. As a consequence, part of the propulsion provided by the swimming cells is lost, leading to a reduction in the instantaneous speed of the aggregate (figures 3c and 4). The instantaneous speed of all aggregates monotonically decreases with additional sperm cells, eventually reaching zero when groups have no net polarization and cannot move. This effect is far more pronounced in sperm with wider heads, because slender cells are able to pack more efficiently with nearly parallel orientations in far larger numbers.

To quantify the relative velocity increase caused by aggregation, we have compared the mean squared displacement of aggregates of different size at a fixed time $t = 10^5$ (figure 3d). Although limited to a specific time, this measure has the benefit of capturing both the advantages of the rotational

![Figure 3](image_url)

**Figure 3.** (a) Trajectories of five different aggregates with $N = 1, 4, 8, 12$ and 16. Upon increasing in size, the aggregates become progressively more persistent, because of the reduced fluctuations in the aggregates average direction ($b/a = 0.2, c/b = 1$). (b) Mean square displacement $\langle R^2(t) \rangle_N$ versus time for the same five aggregates of size $N$. (Inset) same data on logarithmic scale. As characteristic of persistent random walkers, the aggregate crossover from a ballistic regime, where $\langle R^2(t) \rangle_N \sim t^2$, to a diffusive one, where $\langle R^2(t) \rangle_N \sim t$. (c) Instantaneous speed of an aggregate relative to a single cell versus the number of cells in the group for various aspect ratios $b/a$ and $c/a$. This is calculated as the mean square displacement after one time step normalized by the same value for a single cell: i.e. $\langle R^2(1) \rangle_N / \langle R^2(1) \rangle$. Regardless of the aspect ratio, the instantaneous speed of an aggregate decreases monotonically as this becomes more isotropic, hence cells swim against each other. This effect is more pronounced for isotropic head shapes, whereas is barely visible for highly anisotropic cells. (d) Relative mobility increase for an aggregate versus the number of cells in an aggregate, for the same aspect ratio values. This is calculated as the long-time mean square displacement ($t = 10^5$) normalized by the same value for a single cell. The increased persistence owing to aggregation results in a higher mobility of the aggregates. This benefit is offset in larger aggregates as the geometry of the group forces sperm to swim against one another. For these simulations, the adhesive region of the sperm cells is at the tip of the head. (e) Illustration of the head shapes used for (c,d). The parameter values used in the simulations are outlined in §2.
damping and the disadvantage of loss of instantaneous speed. This can then be combined with our direct measurements of the instantaneous speed to give a more complete picture of the behaviour of the aggregate.

We normalize mean squared displacement of the aggregates by that of an individual cell to obtain a relative velocity increase. With this choice of $t$, the optimal number of cells ranges between 7 and 10, with a maximum velocity increase of nearly thirty times. This behaviour exhibits strong dependence on the geometry of the sperm cell head, with slender cells generally exhibiting a far greater increase in distance travelled, which is true even for very large aggregates. The origin of this property is twofold: on the one hand, the instantaneous speed of the aggregate is higher for slender cells, as mentioned earlier. On the other hand, very prolate ellipsoids pack more efficiently than other shapes. This hinders the reorientation of the cells inside an aggregate, thus enhancing the rotational damping described earlier.

3.2. Head geometry strongly affects the performance of the aggregate

In order to shed light on the effect of sperm head geometry on the performance of motile aggregates, we performed simulations for a wide range of head aspect ratios ($b/a$ and $c/a$) and aggregate size ($N$). Figure 5a shows the maximum relative velocity of an aggregate for different aspect ratios. It is immediately clear that the fastest aggregates contain cells with a smaller aspect ratio, owing to the fact that slender cells can form tightly packed aggregates where the velocities of the individual cells are roughly parallel. Interestingly, the strongest dependence observed here is on the second aspect ratio $c/b$, which implies that prolate (i.e. rod-like) and oblate (i.e. plate-like) heads have comparable efficiency upon aggregation. Intuitively, this originates from the fact that plate-like sperm heads are able to stack, thus increasing the alignment of the velocities of individual cells. Furthermore, for a given volume, plate-like heads have a significantly larger area than both spherical and rod-like heads, hence more space for membrane-bound adhesion molecules [10].

Figure 5b shows the optimal size of sperm aggregate for different shaped sperm cells. Consistent with the previous considerations, flatter sperm heads obtain the greatest advantage when they form larger aggregates, this again is owing to their ability to pack many cells effectively with highly aligned velocities. For very spherical sperm heads, there is no advantage to forming an aggregate, and we see that the optimal group size consists of a single cell (blue region in figure 5b).
The structure of the adhesive region strongly affects the performance of the aggregate

The results reported in the previous two sections rely on the assumption that the adhesive portion of the sperm head is localized at the forward tip. In order to study how the structure of the adhesive region affects the performance of the aggregate we have simulated five variants with aspect ratio \( b/a = 0.5 \) and \( c/b = 0.4 \), which is close to features observed in \( P. maniculatus \) and \( Peromyscus polionotus \) [14]. We define the adhesive region as a strip that starts from a position \( s \) from the tip of the sperm head and extends towards the tip over a width \( w \), i.e. when \( s = w \) then the front of the sperm cell is covered in an adhesive cap that extends down to the tip, whereas if \( s > w \) the adhesive region is limited to a band (figure 6b).

Figure 6b shows the typical velocity increase obtained from the simulations of the different variants. The best performance is obtained when the adhesive strip occupies a small portion of the sperm head. Conversely, an excessively large adhesive region can compromise the flexibility of the aggregates, resulting in a breakup of the aggregates. The aggregates in these simulations consist of \( N = 5 \) cells which each have the aspect ratios \( b/a = 0.5 \) and \( c/b = 0.4 \) to approximate the shape of \( Peromyscus \) sperm cells.

3.4. Aggregation performance of \( Peromyscus maniculatus \) from morphometric data

In this section, we extend our approach to account for more realistic head geometries. In particular, we consider the sperm cell of \( P. maniculatus \). The purpose of this analysis is twofold. First, using realistic head geometries allows us to test the validity of the results presented in the previous sections and to demonstrate that many of the properties illustrated with the elliptical model are robust and carry over to realistic head geometries. Second, we explore the interplay between the geometry of the apical hook and the structure of the adhesive region and identify a number of highly efficient configurations that could provide a basis for future experimental studies.

Sperm samples were dissected from the epididymes of sexually mature males, fixed in 2.5% glutaraldehyde, dehydrated first in a graded ethanol series, then in hexamethyldisilazane, and sputter coated in gold/palladium. Samples were imaged on a Hitachi SU-3500 scanning electron microscope at the Laboratory for Biological Ultrastructure, University of Maryland (figure 7a,c). The two planar electron micrographs shown in figure 7a,c were first traced to create a general outline and identify the centre-line of the hook. This was then extruded to a three-dimensional model consisting of 400 triangles (figure 7b).

Sperm aggregates were simulated in small groups such as that imaged in figure 7d. To shed light on the interplay between the hook geometry and the structure of the adhesive region, we considered six different variants as shown in figure 8. The instantaneous speed of the aggregates (figure 8b) is particularly sensitive to the structure of the adhesive region in the presence of the hook. Aggregates with the adhesive region covering only the mid-section or hook are able to maintain consistently high instantaneous speeds, whereas sperm cells where the adhesive region extends beyond the midpoint of the head are significantly slower (figure 8b). At longer time scales the difference between the aggregates is even more pronounced, with sperm cells having an adhesive cap on the forward section of the head being the fastest and sperm cells with the whole body adhesive being the slowest (figure 8c). These behaviours are rooted in the physical mechanism outlined in §3.3. The torques applied by the adhesion molecules are not necessarily promoting alignment unless limited to the upper half of the sperm head. The \( P. maniculatus \) sperm head is substantially flat, with two large roughly parallel sides which reduce the torque exerted by adjacent sperm, allowing for very efficient stacking. This cell shape reduces the crowding
effect and desegregation observed for the ellipsoidal sperm heads, resulting in generally more robust and efficient sperm aggregates.

4. Discussion and conclusion
Using numerical simulations, we have explored how the geometry of the sperm head affects the performance of aggregation. In our simulations, the sperm head is modelled as a persistent random walker characterized by a non-trivial three-dimensional shape. The head is further equipped with an adhesive region, where the cell binds to the head of other cells. Upon modelling the head via a simple parametric shape (i.e. an ellipsoid), we have established that the mobility of sperm aggregates is strongly affected by the head geometry. In particular, slender and oblate head shapes lead to...
significantly faster aggregates compared to spherical head shapes, because they allow the cells to form tightly packed and highly polarized aggregates, thus reducing the spread in the velocities of the individual cells.

The structure of the adhesive region also has a profound impact on both the stability and the swimming performance of the aggregates. In particular, by simulating ellipsoidal heads endowed of adhesive strips of varying thickness and location, we find that some degree of internal flexibility is a vital feature for successful aggregation. When aggregates first form they typically do not have a polar structure and the cells tend to swim against each other. Cells having large adhesive areas are too tightly bound to their neighbours to reorient and eventually break apart. On the other hand, cells with smaller adhesive areas, such as a strip in the vicinity of the head equatorial plane, are sufficiently mobile to reorient in such a way that all the cells move toward the same direction. This enhances the stability of the aggregates as well as the persistence of their random motion.

Finally, most muroid rodents produce sperm with one or more hooks on the head of the cell [10]. Although the function of sperm hooks is unresolved, it has been hypothesized that the hook may interact with the epithelium of the female oviduct to aid sperm migration [31], may offer a hydrodynamic advantage to singly swimming cells [32], and/or may permit cells to attach to the hooks or flagellum of other sperm cells, thereby creating aggregates [11] (but see [33]). In this latter prediction, evidence suggests that sperm–sperm adhesion occurs along the inner surface of the hook in wood mice (A. sylvaticus) [2]. Our model suggests another alternative, that the hook acts as a shield in combination with an adhesive region localized at the acrosome or directly below at the equatorial segment. By shielding one side of the sperm head, the hook could favour the formation of aggregates in which all the cells have the same orientation, leading to more dense packing and a more effective alignment of the velocities of the interacting cells. This hypothesis is consistent with empirical observations of Peromyscus sperm [14] and numerical simulations (figure 8).

Ethics. Wild-derived P. maniculatus bairdi used to harvest sperm for the scanning electron micrographs were obtained from the Peromyscus Genetic Stock Center at the University of South Carolina and were maintained at the University of Maryland (UMD) in accordance with guidelines established by the UMD Institutional Animal Care and Use Committee (approved project: R-15-61 granted to H.S.F.).

Data accessibility. This work did not generate data other than those presented in the article.

Authors’ contributions. D.J.G.P. designed the mathematical model, wrote the code, performed the numerical simulations and analysed the data. L.A.H. wrote the code and performed the numerical simulations. K.A.H. carried out the electron micrographs. H.S.F. conceived and coordinated the study. L.G. designed the mathematical model, conceived, designed and coordinated the study. All authors wrote the manuscript.

Competing interests. We declare we have no competing interests.

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