Efficiency of Initiating Cell Adhesion in Hydrodynamic Flow

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We theoretically investigate the efficiency of initial binding between a receptor-coated sphere and a ligand-coated wall in linear shear flow. The mean first passage time for binding decreases monotonically with increasing shear rate. Above a saturation threshold of the order of a few 100 receptor patches, the binding efficiency is enhanced only weakly by increasing their number and size, but strongly by increasing their height. This explains why white blood cells in the blood flow adhere through receptor patches localized to the tips of microvilli, and why malaria-infected red blood cells form elevated receptor patches (knobs).

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Cohesion in biological systems and biotechnological applications is usually provided by specific bonds between receptors and ligands. The formation of these bonds requires a physical transport process which brings receptors and ligands to sufficient proximity for binding. On the cellular level, one of the most prominent examples is the binding of blood-born cells to the vessel walls under the conditions of hydrodynamic flow. For white blood cells, initial binding to the vessel walls is the first step in their hunt for pathogens, which is then followed by rolling adhesion, firm arrest, and extravasation [1]. Similar processes are used by stem and cancer cells which travel the body with the blood stream. Initiating binding to vessel walls is also essential for malaria-infected red blood cells in order to avoid clearance by the spleen and possibly also to foster rupture and release of new parasites into the blood stream [2]. Similar questions about initial binding under flow conditions arise for bacteria, e.g., when binding to the intestinal wall [3], and in biotechnological applications, e.g., for adhesion-based cell sorting [4]. In order to control shear flow and cell density in a quantitative way, standard setups are flow chambers [5]. An essential but largely unexplored aspect of these processes is receptor geometry, that is, size, height, and separation distance of the receptor patches. One way to address this issue experimentally is the use of receptor-coated beads [6].

Earlier theoretical efforts in this context have been focused mainly on issues related to white blood cells, including modeling of the initiation of adhesion at high cell densities (e.g., due to hydrodynamic interactions) [7] and the process of rolling adhesion [8]. In these studies, the parameters characterizing receptor-ligand binding are usually fixed at physiologically motivated values. In this Letter, we take a more general view and ask how a variable receptor geometry affects cell capture in hydrodynamic flow. In order to study this issue in a systematic way, we spatially resolve receptors and ligands. The cell is modeled as a rigid spherical Brownian particle in linear shear flow carrying receptors for ligands covering a planar boundary wall. In the absence of interactions with other particles or external forces, there is no reason for such a particle to drift towards the wall and initial binding has to rely completely on thermal diffusion. In order to arrive at a generic model, we consider the simplest of the possible downward driving forces in experiments with flow chambers, namely, gravity. The particle is set free at a certain height above the wall and we calculate the mean first passage time (MFPT) for the first receptor-ligand encounter as a measure for the efficiency of initial cell binding. We consider three models of increasing complexity in regard to the spatial distribution of receptors and ligands. We first show that if the receptors on the cell and the ligands on the substrate are distributed homogeneously, then the corresponding MFPTs can be calculated exactly. In the case that receptors are spatially resolved, we use extensive computer simulations to calculate the MFPTs as a function of their number and spatial dimensions, both in two and three dimensions. As a third case, we in addition consider spatially resolved ligand distribution.

Figure 1 introduces the parameters of our model. We consider a sphere of radius $R$ which moves with the hydrodynamic flow in positive $x$ direction at a height $z$ above the wall with normal $\vec{e}_z$. The simplest possible flow pattern is linear shear flow with shear rate $\dot{\gamma}$. With the no-slip boundary condition at the wall, the unperturbed velocity profile reads $u^0 = \dot{\gamma} z \vec{e}_z$. For a typical cell radius $R = 5 \mu m$ and a typical shear rate $\dot{\gamma} = 100 \ Hz$, the Reynolds number in aqueous solution is well below 1 and the hydrodynamic flow is essentially described by the Stokes equation for incompressible fluids. This is even more true for smaller particles like micron-sized beads. Scaling estimates also show that for typical parameter values for cell elasticity, deformations due to shear flow and lubrication forces are small and therefore the spherical approximation is justified. In addition to the hydrodynamic forces, in our model there are also gravitational and thermal forces acting on the particle. Such a combination of forces is the subject of Stokesian dynamics [9] and in our case leads to the follow-
Ftional degrees of freedom. The mobility matrix
shear force
4
Nr
studied here: (a) homogeneous coverage of cell and wall, (b)
the superscript
Stratonovich sense. The matrix
mobility matrix
Pe´clet number in
troduces another dimensionless number, which we call the
motion dominate, respectively. The gravitational force in-
the limits
z
wall at a height
z
PRL
3
Pe´clet number in
.../3)e_z with some density difference
. The subscript
r
interact with each other through
the thermal force
is assumed to be Gaussian:
7
0
\langle g_{i} \rangle = 0, \quad \langle g_{i} g_{j} \rangle = 2k_B T_r \delta \langle t - t' \rangle. \tag{2}

The superscript S for the multiplicative noise term in
Eq. (1) indicates that it has to be interpreted in the usual
Stratonovich sense. The matrix B in Eq. (1) is related to
the mobility matrix M through \( M = BB^T \). Vectors in Eq. (1)
are six dimensional, representing the spatial and orienta-
tional degrees of freedom. The mobility matrix \( M \) and the
shear force \( F_s \) for a spherical particle above a wall cannot
be obtained in analytically closed form. However, they can
be calculated numerically to high accuracy and we will use
this for our simulations [11].

Considering the physical dimensions of our problem shows
that the motion of the cell is essentially governed by two-dimensionless numbers. For length, the natural
scale is cell radius \( R \). Mobility and shear force scale as
\( M \equiv 1/(6\pi \eta R) \) and \( F_s = 6\pi \eta R^2 \gamma \), respectively.
For time, there are two relevant time scales, the deterministic
time scale \( 1/\gamma \) and the diffusive time scale
\( R^2/D = 6\pi \eta R^3/k_B T_a \), where we have used the Einstein relation
\( D = Mk_B T_a \) for the diffusion constant \( D \). Therefore,
the relative importance of hydrodynamic to thermal motion is
described by the Péclet number \( Pe = 6\pi \eta R^3/\gamma/k_B T_a \). In
the limits \( Pe \rightarrow 0 \) and \( Pe \rightarrow \infty \), diffusive and deterministic
motion dominate, respectively. The gravitational force
introduces another dimensionless number, which we call the
Péclet number in \( z \) direction, \( Pe_z = FGR/k_B T_a = 4\pi g \Delta \rho R^4/(3k_B T_a) \). In the following, we will nondimen-
sionalize length and time by \( R \) and \( 6\pi \eta R^3/k_B T_a \), respectively.

We start by considering homogeneous coverage of cell and
wall with receptors and ligands, respectively, compare
Fig. 1(a). Then rotational degrees of freedom are irrelevant.
Because the wall breaks the symmetry only in the \( z \) direc-
tion, motion in the \( x-y \) plane is decoupled from our problem.
Thus in this case we essentially deal with a MFPT in
one dimension, which is independent of shear rate \( \gamma \) and
which can be approached with standard methods for the
appropriate Fokker-Planck equation. Binding is identified
with approach of receptor and ligand to a capture distance
\( r_0 \). Applied to the case of homogeneous coverage, the cell
has to fall to the capture height \( 1 + r_0 \). If dropped from the
initial height \( z_0 \), the respective MFPT can be shown to be
\( T_h = \frac{1}{Pe_z} \int_{z_0}^{z_0 + r_0} dz \frac{1}{M_{zz}(z)} \). \tag{3}\n
Thus the MFPT scales inversely with the gravitational
force driving the cell onto the wall. With the lubrication
approximation \( M_{zz}(z) = 1 - 1/z \) we find
\( T_h = \frac{1}{Pe_z} \left[ z_0 - 1 - r_0 + \ln \left( \frac{z_0 - 1}{r_0} \right) \right] \). \tag{4}\n
Thus the MFPT diverges logarithmically with vanishing
capture distance \( r_0 \) (that is, when the cell has to get
infinitely close to the wall) and linearly with initial height
\( z_0 \) (that is, when the cell starts infinitely far away from the
wall). Although only the constant force chosen here results
in an analytical result like Eq. (4), for other types of force
laws it is straightforward to numerically calculate corre-
responding falling times \( T_h \) [10].

We next consider the case of a spatially resolved recep-
tor distribution, compare Fig. 1(b). Now the cell is equi-
distantly covered with \( N_r \) receptor patches, each with
radius \( r_p \) and height \( r_0 \). We first note that in this case,
initial orientation becomes important. Moreover, now
shear rate \( \gamma \) enters the analysis: the shear flow increases
cell rotation, and for heterogeneous receptor coverage, this
strongly influences when the first receptor can bind the first
ligand. Because experimentally it is hardly possible to
prepare the initial orientation of the cell, in the following
we average over all possible initial orientations. One can
show that the angle-averaged MFPT is the MFPT to fall
from initial height \( z_0 \) to some intermediate height \( z_m \)
according to Eq. (3) (that is independent of orientation)
plus the angle-averaged MFPT to bind from the initial
height \( z_m \). In this sense, the initial height is not relevant
for our problem and in the following we always use \( z_0 = 2 \),
that is the cell has to fall for the distance of one radius
before binding can occur.

In Figs. 2(a) and 2(b) we show the MFPT as obtained by
extensive computer simulations as a function of Péclet
number \( Pe \approx \gamma \) and receptor patch number \( N_r \) for two
(2D) and three dimensions (3D), respectively. Here 2D
FIG. 2 (color online). Effect of shear rate on mean first passage time $T$ for initial binding for spatially resolved receptors in (a) two (2D) and (b) three dimensions (3D). The scales for length and time are $R$ and $6\pi \eta R^3/k_BT_a$, respectively. The Péclet number $Pe = 6\pi \eta R^3g/k_BT_a$ and the Péclet number in $z$ direction $Pe_z = 4\pi gD\rho R^3/(3k_BT_a)$ represent the strengths of the hydrodynamic and gravitational forces, respectively, relative to the thermal forces. $z_0 = 2$, $Pe_z = 50$, $r_0 = r_p = 10^{-3}$.

means that translational motion is restricted to the $x$-$z$ plane and rotations are restricted about the $y$ axis, which allows for much faster simulations. Each data point in Fig. 2 is the average of at least $10^5$ simulated trajectories of the Langevin equation [Eq. (1)]. Our simulations are very time consuming because with the receptor patches we resolve objects of typical size $10^{-3} \text{nm}$, that is, nm-sized patches on micron-sized cells. From Fig. 2 we first note that $T$ decreases monotonously with increasing $Pe$ and that the shear rate does not change the relative sequence of the curves for different $N_r$. Thus the larger shear rate and the more receptor patches present, the more efficient cell capture. The crossover between the diffusion- and convection-dominated regimes does not occur at $Pe = 1$, but at much larger values $Pe = 10^2$. Next we note that in the 2D case [Fig. 2(a)], for large $Pe$ or large $N_r$, all curves level off to the exact result for homogeneous coverage from Eq. (3), because in these two limits, the binding process effectively becomes rotationally invariant. In the 3D case [Fig. 2(b)], the homogeneous reference value is only achieved for large receptor numbers. The reason is that for small numbers of receptors, the cell might have to rotate around the $x$ axis before a receptor moving on a circle parallel to the $x$-$z$ plane is able to bind a ligand on the wall. Therefore, in 3D thermal diffusion remains essential even in the case of large Péclet number.

In order to achieve a better understanding of the simulation results shown in Fig. 2, it is instructive to decompose the process into periods of falling and rotation, respectively. A detailed analysis shows that in the 2D case this decomposition allows to derive scaling laws for different limits in regard to $Pe$ and $Pe_z$. An important case is the one of large $Pe_z$, when the cell is strongly driven onto the wall. Then the binding process can be decomposed into a initial falling period described by Eq. (3), followed by a purely rotational search for ligand, which is independent of $Pe_z$ and can be calculated analytically:

$$T_r = A_0 \Delta \theta^2 \coth\left(\frac{\Delta \theta}{2\theta_p}\right) - 2D_\theta \Delta \theta \frac{2A_0^2}{\theta_p^2}.$$  

Here $\Delta \theta = \theta_i$ is the angle between the absorbing boundaries, $\theta_i = 2\pi/N_r$ the angle between receptor patches, $A_0 = Pe/2$ the rotational drift, and $D_\theta = 3/4$ the rotational diffusion constant. From Eq. (5) we get $T \sim 1/N_r^2$ and $T \sim 1/(N_r Pe)$ for small and large $Pe$, respectively, in excellent agreement with the scaling found in our simulations. In general, Eq. (5) is a good qualitative description of the 2D data shown in Fig. 2.

In order to consider the case of spatially resolved ligand, compare Fig. 1(c), we cover the boundary wall with a square lattice of circular ligand patches, with lattice constant $d$ and patch radius $r_d$. Typically the saturation threshold for the parameters used is located at a mean patch-to-patch distance $d$ of about 0.17, both in regard with receptors and ligands. For a coverage below the threshold, Fig. 3 shows for this case that the MFPT saturates both when increasing receptor coverage by increasing $N_r$ or ligand coverage by decreasing $d$. A similar saturation behavior is also found when increasing receptor and ligand patch sizes $r_p$ and $r_d$. The dashed lines show the scaling behaviors $T \sim 1/N_r$ and $T \sim 1/r_l$ in regard to receptor and ligand coverage, respectively, where $d$ represents the distance between receptor and ligand patches, respectively. This can be understood by noting that the 1D MFPT for capture by diffusion scales $\sim d^2$ where $d$ is the distance between the two absorbing boundaries. The saturation effect observed with respect to $N_r$, $\rho_l$, $r_p$, and $r_d$ results from the space-filling nature of diffusion which has been implicated before for the efficiency of ligand capture by a cell [12].

![Image](138103-3)
We first note that the MFPT is much more influenced by a change in the receptor patch radius \( r_p \) for \( r_0 = 10^{-3} \) and \( r_0 = 10^{-2} \) as well as for three different values of \( N_r \). We finally turn to the effect of the capture distance \( r_0 \). In Fig. 4 we show the MFPT in the diffusive limit as a function of the receptor patch radius \( r_p \) for \( r_0 = 10^{-3} \) and \( r_0 = 10^{-2} \). The lines in Fig. 4 show that this equation can be fitted extremely well to the data for homogeneous coverage from Eq. (3) has been subtracted. The lines are fits according to Eq. (6). Other parameters as in Fig. 2.

We finally turn to the effect of the capture distance \( r_0 \). In Fig. 4 we show the MFPT in the diffusive limit as a function of the receptor patch radius \( r_p \) for \( r_0 = 10^{-3} \) and \( r_0 = 10^{-2} \) as well as for three different values of \( N_r \). We first note that the MFPT is much more influenced by a change in \( r_0 \) or \( N_r \) than by a change in \( r_p \). One can show on geometrical grounds that the two parameters \( r_0 \) and \( r_p \) conspire to define an effective receptor patch size \( \sqrt{r_0} + r_p \) which then in turn determines the probability for binding. This line of reasoning leads to the formula

\[
T = \frac{a}{b + r_p} + T_h, \tag{6}
\]

where \( a = 2t_d/\sqrt{r_0}, b = \sqrt{r_0}/2, t_d \) is a typical diffusion time between binding attempts which scales \( \sim 1/N_r \), and \( T_h \) is the homogeneous result from Eq. (3). Equation (6) implies that even for vanishing receptor size the MFPT remains finite due to a finite \( r_0 \). The lines in Fig. 4 show that this equation can be fitted extremely well to the simulation results. Moreover, the fitted values for \( a \) and \( b \) agree roughly with the predicted scaling behavior.

In summary, our results show that the efficiency for initiating cell adhesion in hydrodynamic flow is strongly enhanced by increasing the number of receptor patches \( N_r \), but only up to a saturation threshold. An increase of patch size \( r_p \) leads only to a weak enhancement of binding efficiency. In contrast, a strong enhancement results from increasing the patch height \( r_0 \). For example, for a few hundred receptor patches, an elevation of \( r_0 = 10^{-2} \) makes initial binding already as efficient as for a homogeneously covered cell. Strikingly, white blood cells are indeed characterized by such a receptor geometry, because they are covered with hundreds of protrusions (microvilli, typical height \( \sim 300 \) nm, corresponding to \( r_0 = 0.06 \)) which carry adhesion receptors like \( L \)-selectin at their narrow tips [5]. In general, white blood cells operate in the limit of a homogeneously covered cell not only due to their receptor geometry, but also because during capture they are usually exposed to environments with \( Pe = 10^4 \)–\( 10^5 \) (a typical value for \( Pe \) is \( 300 \)). The principle of enhancing capture efficiency by elevation of receptor patches seems to be also used by other biological systems. One example of large medical relevance appears to be malaria-infected red blood cells, which develop thousands of little adhesive protrusions (knobs, typical height \( 20 \) nm, corresponding to \( r_0 = 0.004 \)) [2]. The results presented here do not only allow to understand the efficiency of cell capture in these biological systems in a unified way, but can also be used for developing corresponding applications in biotechnology, including adhesion-based cell or particle sorting.

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[10] A detailed derivation of the Langevin equation and more details of our simulation algorithms and results will be published elsewhere.