Modeling cell interactions under flow

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Abstract In this review, we summarize the current state of understanding of the processes by which leukocytes, and other cells, such as tumor cells interact with the endothelium under various blood flow conditions. It is shown that the interactions are influenced by cell–cell adhesion properties, shear stresses due to the flow field and can also be modified by the cells microrheological properties. Different adhesion proteins are known to be involved leading to particular mechanisms by which interactions take place during inflammation or metastasis. Cell rolling, spreading, migration are discussed, as well as the effect of flow conditions on these mechanisms, including microfluidic effects. Several mathematical models proposed in recent years capturing the essential features of such interaction mechanisms are reviewed. Finally, we present a

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recent model in which the adhesion is given by a kinetics theory based model and the cell itself is modelled as a viscoelastic drop. Qualitative agreement is found between the predictions of this model and *in vitro* experiments.

Keywords Adhesion Kinetics theory · Flow conditions · Cell rheology

Mathematics Subject Classification (2000) 65N30 · 70F35 · 76A10 · 92C17 · 92C45

1 Introduction

For several years, many researchers have devoted considerable effort to understand the interactions between cells and the endothelium occuring as the former travel through the blood stream, especially during inflammation or cancer metastasis. Cell–cell interactions are essential for circulating cells to make contact with the vessel walls (since a cell simply moves away from the wall in a pressure driven flow when no interactions exist) and eventually penetrate through the endothelial barrier [96,57]. This process is called extravasation and is influenced by several important factors:

- Cell adhesion properties and cell signaling
- Flow conditions
- Capillary geometry or the role of confinement
- Cell's rheological properties

The mechanisms involved in the above processes are usually divided into several steps. Cells (i.e. leukocytes or cancer cells) first interact with the endothelium through the formation of weak bonds related to specific adhesion molecules, thus resulting in the rolling process. This process is well understood for leukocytes but is still under investigation for cancer cells. After this initial step, stronger bonds are required for the cell to be arrested [68]. Then cells are able to resist the flow, spread and migrate to reach potential sites where they are able to extravasate. This can happen either at cell–cell junctions [27] or directly through endothelial cells [79]. A typical sketch of the mechanisms is shown in Figure 1. This figure also shows the relevant adhesion molecules for each stage. These adhesion molecules belong to four different superfamilies, namely selectins, integrins, immunoglobulins and cadherins.

We next describe the effect of shear flow on the migration of cells. It is known that circulating cells are usually located near the center of blood vessels. During inflammation neutrophils interact with erythrocytes and drift closer to the endothelium, a process known as margination [52,83], where interactions with the endothelium become more effective. This process is not well understood and seems to be due to RBCs aggregation tending to push neutrophils closer to the endothelium while the shear rate is lowered due to the presence of RBCs [83]. Furthermore, margination is also affected by cytokines which induce an increase in neutrophils migration. Finally, the confinement (in small capillaries) enhances the frequency of interaction between neutrophils and the endothelium.

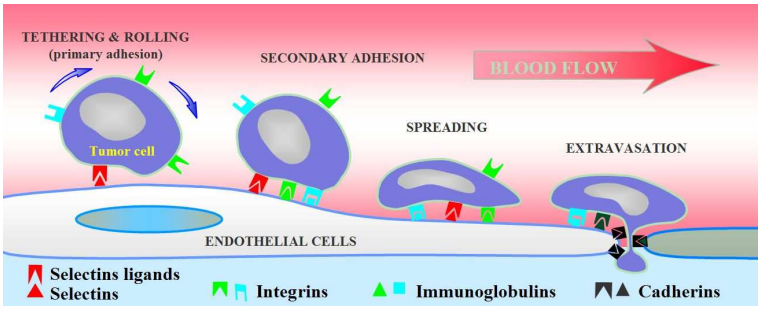

Fig. 1 Mechanisms involved in cell transmigration under flow. Identification of the major adhesion molecules.

In light of recent studies, the influence of the flow field on adhering cells under physiological conditions (in typical post–capillary venules shear stresses are between $0.1 - 20\,dyn/cm^2$ or $0.01 - 2\,Pa$) is better understood. The effect of flow on adherent cells such as neutrophils can lead to either retraction of pseudopods [74,80] or the opposite [28] in the case of passive leukocytes. This means that depending on the state of cells' cytoskeleton, different cells behave differently because their microrheological properties are controlled by the cytoskeleton.

There are differences between leukocytes and cancer cells, some of which arise due to the fact that leukocytes are smaller and more motile. Also the mechanisms involved during transmigration suggest that cancer cells have smaller rigidities as compared to leukocytes [70,88]. Modelling this problem is therefore a considerably difficult task, as it requires taking into account all of the above effects.

Cell–cell adhesion has been considered previously in terms of energy landscapes [8] to explain how bonds can be broken when subjected to a force, such as that arising from hydrodynamic shear stress. This has lead to the development of theories for the rate–dependence of forces, bond lifetimes and rolling velocities, and experiments have been performed to verify these theories for example using micropipettes [36] and AFM [76,39]. The rolling of cells is a subject of particular interest and has been studied extensively both experimentally and theoretically. In particular, it has been postulated recently that bonds between molecules can exhibit different mechanical behaviors when stressed, responsible for the tethering and rolling of leukocytes [76]. Such bonds can either show a shorter lifetime when subjected to mechanical forces, or exhibit a prolonged lifetime when possible interlocking between molecules occurs (i.e. the so–called "catch–bonds"). The debate on this issue is still ongoing [20], and is critical for addressing the major question of the threshold effect, i.e. the minimum level of shear rate necessary to achieve cell rolling [23]. Applications to cell rolling problems have been considered from different points of views. For example, adhesion phenomena have been treated in idealized situations where cells adhere to a wall. One can model the attraction or repulsion using a simple potential energy [97] corresponding to attraction when cells are not to close. As a cell comes closer to

the wall, a repulsive force acts such that it cannot get closer than a critical distance. Another way to model cell adhesion is to consider the associated kinetics equation leading to bonds formation and dissociation (forward and reverse reactions between receptor and ligand molecules). This allows to predict the bond density and therefore the force necessary for cell detachment [31,46] but other models like stochastic ones are also of interest, relying on predictions based on probabilistic events [42,103].

To model adhesive forces accurately, it is necessary to account for the cell flattening under flow, since it leads to the formation of an increased number of bonds [32]. Therefore in recent more realistic models the cell is described as a composite drop [82] with a nucleus and a cytoplasm or as a capsule with a viscous fluid surrounded by a membrane [6,66,55]. The literature about membranes is quite rich and also relevant for modelling vesicles (viscous fluid with a membrane allowing for bending effects only, together with a conserved area) [12,90], which are more suitable models of red blood cells (RBCs). In recent years, the deformation of cells has been simulated numerically using these more realistic models, employing several different numerical approaches [82,62,56], leading to quite realistic comparisons with experimental data for leukocytes.

In this paper, we will review the main issues important for the understanding of cell–endothelium interactions. In Sect. 2, we analyze the different adhesion proteins and signalling events taking place during cell transmigration. In Sect. 3, the effect of the flow on cell rolling is investigated as well as its influence on cell adhesion in view of our recent experiments. Possible explanations are presented. Then we describe models leading to kinetic adhesion theories [8,47,31] which have been used extensively (Sec. 4). Finally, the importance of cell deformability described by several viscoelastic cell models is discussed (Sec. 5). Some of the results obtained using a model proposed by us for investigating the rolling behavior of a composite viscoelastic cell under flow [56] are described.

2 Adhesion molecules involved in cellular interactions

In most cellular systems, cells are subjected to different forces giving rise to a competition between repulsion effects (electrostatic, steric ones) and attractive effects (specific bonding), also influenced by the presence of long polymeric macromolecules called adhesion molecules. Thermodynamic theories offer an interesting basis to study this competition [9] and allow one to predict how two cells get in contact, deform and adhere to each other. Cells are usually surrounded by a glycocalyx layer (typically $5 - 10\,nm$) which is thin enough for macromolecules to penetrate and create stable links. These links or bonds can become quite strong, especially when they involve mechanical interlocking, and are often named **receptor–ligand** bonds. Individual bonds come from a combination of van der Waals attraction, hydrophobic contact and hydrogen bonding, thus leading to a high binding energy, and require conformational recognition by both receptor and ligand molecules (or macromolecules). Receptors are usually transmembrane proteins, also named **Cell Adhesion Molecules** (CAMs) usually divided into several families or

superfamilies. These families of molecules are responsible for the formation of cell–cell junctions, cell recognition, migration, growth, metabolism and cell signalling.

2.1 Adhesion molecules superfamilies

The four main families of molecules are shown in table 1 and have the following properties [84,71,25] :

- Cadherins (E–Cadherins, etc.). They interact with themselves (homotypic bonds) and create strong bonds like in the case of cell–cell junctions (for example at the junctions between the endothelial cells making the vascular wall). E–Cadherins are important as their down–regulation seems to be responsible for the loss of adhesion involved when tumor cells escape from an initial tumor [81] to become invasive. Differential expression of Cadherins is also involved during cell segregation into tissues [40].
- Integrins. They form heterotypic bonds usually with the immunoglobulins molecules or the Extra–Cellular Matrix (ECM). Typical integrins present on the leukocyte (LFA–1 or Mac–1) or tumor cell (Figure 1) interact with immunoglobulins such as ICAM–1 or PECAM–1 on the endothelial cells side and form strong bonds responsible for cell arrest. Integrins are composed of two subunits (α and β chains) enabling to modify their conformation to adapt to the ligand, for example when intracellular signals make them switch from an inactive form to an active one. By interacting with the ECM, integrins are particularly involved in cell migration [41].
- Immunoglobulins. They can form both heterotypic and homotypic bonds. For example N–CAM binds with N–CAM and similarly PECAM–1 binds with PECAM–1. On the other hand VCAM–1 (an immunoglobulin) binds with VLA–4 of the integrin family. These bonds, as indicated above, can lead to firm cell–cell adhesion.
- Selectins. They are heterotypic molecules. P–selectin and E–selectin form bonds with glycoprotein PSGL–1, ESL–1 and CD44 (carbohydrate sites on the leukocytes) whereas L–selectin interacts with CD34. Such interactions are responsible for the rolling of leukocytes, a prerequisite for the activation of integrin bonds [68]. The mechanical anchorage of P–selectins with PSGL–1 seems to be responsible for the "catch–bond" effect [76].

2.2 Interactions at the cell–cell level and signalling

As discussed earlier, at the molecular level, adhesion proteins are responsible for the receptor–ligand bonds which lead to microscopic adhesion at the cellular scale. Cooperative effects are necessary to obtain higher levels of forces [39,102] and a simplified cooperative model indicates that such effects can be additive [39,17]. This can be measured by different techniques such as optical tweezers, AFM, the Surface Force Apparatus (SFA) and micropipettes [69]. To sum up, typical bonds are in the range $5 - 100\,pN$ [78]

Table 1 Main adhesion molecule superfamilies with description and interactions. Examples of molecules involved in cell transmigration are indicated in parenthesis.

Molecule family	Type of bonds formed	Associated receptors
Cadherins (E-Cadh, N–Cadh)	Homotypic	Cadherins (E-Cadh, N–Cadh)
Integrins (LFA–1, Mac–1)	Heterotypic	Immunoglobulins, ECM
Immunoglobulins (ICAM–1, PECAM–1)	Homo/Heterotypic	Immunoglobulins/Integrins
Selectins (E-selectin, P–selectin)	Heterotypic	Glycoproteins (PSGL–1)

depending on loading rates, but cell–cell contacts requiring the recruitment of interconnected multiple bonds may range up to $0.1 - 10\,nN$, depending on cell contact area [10]. Cells in contact with a substrate form focal contacts [11] whose size and number increase with a stiffening of the matrix elasticity [24]. Such focal contacts (around $1\mu m^2$ in size) usually develop forces in the range of $5\,nN/\mu m^2$ representing about 250 molecular bonds. These contacts contain mainly proteins of the integrin type linked to molecular clusters containing many intracellular proteins (FaK, α–actinin, vinculin, talin, paxilin) connecting these areas with the cytoskeleton. Thus cells can use molecular clusters to exert traction forces on the substrate. When different cells are connected with each others, it is likely that the breaking of bonds can be done either in a cooperative or independent way, in a stochastic manner. Kinetic models of bond breakup and formation will therefore be presented in Sec. 4. Cell adhesion molecules also play an important role in the structure of tissues through mechanical interactions, and permit communications between adjacent cells.

As soon as cells are in contact, signalling events take place. This, for example, occurs during the first leukocyte–endothelial cell interaction (rolling for example). Adhesion proteins then play the role of mechanical transducers producing outside–in signals, and signals induce the activation of proteins required to make firm bonds or to reinforce adhesion sites [2]. Signalling also takes place through smaller soluble molecules (cytokines, growth factors) released by cells acting over long distances. Together with CAMs, the complete picture leads to arrays of signalling pathways whose complexity is still under investigation, but it is understood that they influence the mechanisms governing cell–cell interactions, deformation and migration.

3 Influence of flow on cell motion

Many studies have attempted to analyze the influence of blood flow on the motion and interactions among cells. Blood is constituted mainly of red blood cells (RBCs), platelets and a few leukocytes (neutrophils, eosinophils, mono-

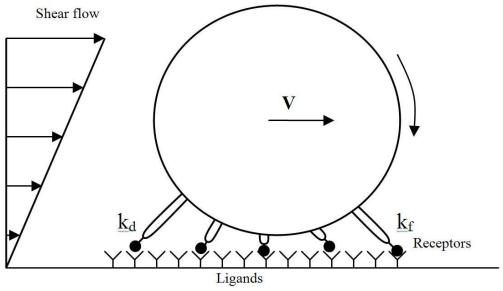

Fig. 2 Sketch of the motion of a cell rolling at velocity V with formation and dissociation of receptor–ligand bonds. Receptors are assumed to be located at the end of tethers (redrawn from [23]).

cytes, lymphocytes or basophils) contained within the blood plasma. Usually there is ratio of 1 : 700 of leukocytes as compared to red blood cells. Blood is known to be a Yield stress fluid, and so it can flow only when shear stresses are strong enough to break interactions between cells, such as 'rouleaux' structures formed by RBCs through cell aggregation. Interactions between cells can therefore lead to a rather complex rheology [100] and contact between circulating cells and the endothelium are only possible under certain circumstances, like during inflammation, or in cancer metastasis.

3.1 Rolling motion

During inflammation, a larger number of leukocytes are recruited at the inflammation sites. The process of margination relies on the separation of RBCs and leukocytes, which leads to the relocation of leukocytes close to the vascular wall. Inflammation cytokines are responsible for the expression of E–selectins and P–selectins on the endothelium, interacting with their ligands (Fig.1), i.e. carbohydrate receptors on the leukocyte membrane. Thus the rolling motion of the marginated leukocytes can take place. This phenomenon has been observed *in vivo* using techniques like intravital microscopy in rat mesentery venules [52] and has been combined with confocal microscopy to study cancer micrometastasis [53].

In vitro experiments have been carried out to investigate the rolling of leukocytes on functionalized surfaces [23] (E or P–selectin coated surfaces) or along an endothelium monolayer [25]. Such studies are achieved in flow chambers allowing simultaneous microscopic observations. The flow rates and pressure drops can usually be monitored as well [26]. Physiological conditions encountered in post–capillary venules, are also achieved in these experiments.

When subjected to a flow field, corresponding to an almost linear gradient [61] close to the wall, circulating cells exhibit adhesive transient interactions with the selectin–coated wall. They roll, with the center of mass velocity V, by rapidly forming new receptor–ligand bonds at the front and breaking them

at the rear. In fact, reactions between ligands and receptors seem to happen at the end of tethers or microvilli (typical diameter $0.1\mu m$), as sketched in Figure 2. The rolling velocities V are in the range of a few $\mu m/s$ [68], whereas blood velocity is in the range of $10 - 10^3 \mu m/s$. Because rolling velocities are small, the expression of chemoattractants on the endothelial side is possible, leading to activation of the heterotrimeric G–protein which drives the activation of integrins until the cell is arrested [96]. There is a critical phenomenon, namely, the shear threshold effect, which occurs at low levels of shear stresses [23]. Cells like leukocytes are unable to roll at small shear stresses. Above this critical level, cells begin to roll with a velocity approximately independent of the shear stress. According to these authors it seems that the wall shear rate increases the rate of dissociation of receptor–ligand bonds but this effect is compensated by an increase of the number of bonds thus resulting in a slowly varying rolling velocity as a function of shear rate [4], in agreement with Bell's model [8] (see Sec. 4.1). Rolling on a surface coated with L–selectins is much faster than on those coated with E–selectins and P–selectins. This can be explained in terms of kinetics of dissociation but also in terms of compliance, i.e. the sensitivity of the dissociation with respect to the applied force [3]. Transient behaviors have been observed by measuring rolling velocities undergoing rapid fluctuations [3, 56] due to the fact that few tethers are necessary to achieve cell attachment, and that binding is quite fast, i.e. happens in a second or less. Investigating the effect of P–selectin densities during rolling of leukocytes, Alon and coauthors [4] also observed a linear dependence of the number of bond cells per unit area as a function of time and confirmed that the receptor–ligand bond formation is a first order reaction.

3.2 Effects of flow on an adhering cell

Several different approaches have been used to investigate the influence of the fluid shear stress on adherent cells, both *in vivo* and *in vitro*. Two types of responses have been observed.

- Neutrophils adherent to the wall of a fluid chamber retract their surface projections when subjected to a laminar shear flow ($5\, dyn/cm^2$ or $0.5\, Pa$) [74]. Applying fluid shear stress ($0.4\, dyn/cm^2$ or $0.04\, Pa$) with a pipette on an adherent leukocyte also has the same effect [80]. Cessation of blood flow in a microvessel leads to the emission of pseudopods by leukocytes, which retract when flow is restored [80]. When flow is applied locally onto the lamellipodium, cell protrusions also stop very abruptly in the region of hydrodynamic load ($10\, dyn/cm^2$ or $1\, Pa$) [14]. Mechanical stress generated by cyclic variations of the substrate area [34] seems to make the cell volume smaller.
- An opposite response has also been observed: individual adherent leukocytes respond to the flow applied by a micropipette (corresponding to shear stresses between $0.2 - 4\, dyn/cm^2$ or $0.02 - 0.4\, Pa$) by projecting pseudopods and spreading on the coverglass [28].

The differences observed in response to a fluid shear stress may be due to the initial state of the cells: passively adherent [28] (i.e. round cells not adhering specifically to the substrate), or actively adherent [80] (cells already showing protrusions). The role of the cytoskeleton in a cell's response to fluid shear is also ambiguous. Treating cells with actin-disrupting agent and myosin inhibitors demonstrate that the spreading response to shear stress application requires an intact cytoskeleton and active myosin filaments. The glycocalyx could be washed away by the fluid, revealing previously hidden receptors and sites for adhesion, and also reduce the electrostatic, osmotic and steric forces between the cell and the substrate, thus enhancing spreading. For the retracting case, the effect of the flow on the cytoskeleton is not as clear. Fluid stress leads to F-actin breakdown [80], whereas chemical composition in the stressed region is not altered and actin polymerization is not abolished by the flow [14]. For cells stressed mechanically, F-actin organization is modified with the appearance of radial stress fibers converging to the actin–rich center [34]. The cause of this cytoskeleton alteration is not well understood. On the one hand, the rapidity of the cell response (in the range of seconds, which is not enough for protein synthesis [80]) suggests that some signal transmission occurs across the cell membrane via ionic channels. On the other hand, the small GTPases of the Rho family may play a role in the shear-induced signalling pathway [74]. Finally, this alteration could still be a result of a purely mechanical response.

Experiments with cells adhering to microchannel walls coated with different fibronectin concentrations and subjected to flow show that ligand density strengthens cell adhesion. This is consistent with the slower detachment of an adherent cell by a fluid flow due to an increase in the ligand density [19]. The influence of the shear intensity has also been studied. An increased fluid shear stress leads to a larger cell deformation leading to a faster detachment, in agreement with other studies where the percentage of detached cells is higher at higher shear stresses [72]. The dynamics of cell detachment seems to be progressive, following a peeling process [31,32,30], as observed in side–view microchannels [19].

The role of confinement has not been fully understood. In fact, in small microvessels for which the channel and cell dimensions are of the same order, the flow can considerably alter the behavior of adherent cells subjected to shear stresses. To mimic the microvessel environment, we performed experiments in our lab using a microfluidic device. After inserting cells in the microfluidic parallelepipedic functionalized chamber (fibronectin, $20\,\mu g/mL$ for $1h$), we allowed them to sediment and adhere on the wall and then they were subjected to an increasing shear stress σ (Fig.3). It appears that cell area first increases with time at low shear stresses ($\sigma < 20\,dyn/cm^2$), then exhibits a maximum and finally decreases at high shear stresses ($\sigma > 210\,dyn/cm^2$) as shown in Figure 4. The positive linear slope of the "area-time" plot at low flow rate means that an adherent cell responds to the flow by spreading on the substrate, which is in agreement with the previous observations of passively adherent cells [28]. This probably allows the cell to reinforce its anchoring to counterbalance the drag force due to the flow. Thus an adherent cell can resist the flow by increasing its area. The maximum of the cell area observed

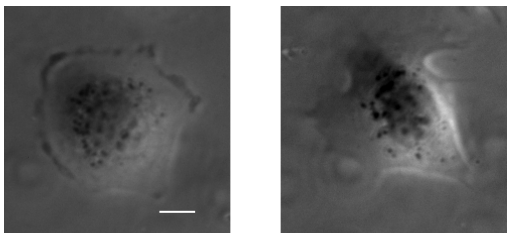

Fig. 3 Cell subjected to an increasing shear flow in our experimental microfluidic chamber. Walls are coated with fibronectin ($20\,\mu g/mL$ for $1h$). Left $\sigma = 0.7\,dyn/cm^2 = 0.07\,Pa$, right $\sigma = 350\,dyn/cm^2 = 35\,Pa$. The flow direction is from right to left. Scale bar is $10\mu m$.

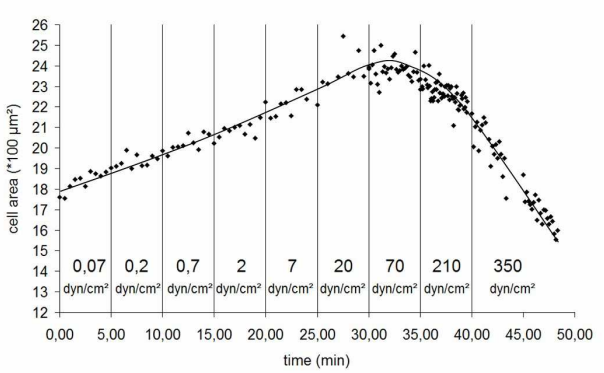

Fig. 4 Evolution of the area of a cell subjected to an increasing shear stress in our microfluidic channel (height $\sim 370\mu m$ and width $\sim 950\mu m$). The line is to guide the eye.

could correspond to an equilibrium where the cell–substrate adhesion torque exactly equilibrates the torque exerted by the flow on the cell. For higher shear stress values, the anchoring torque is not strong enough and the cell cannot resist the flow, its area begins to decrease until detachment from the substrate.

Further studies are under way to link the observed cell response to the mechanisms governing adhesion, spreading and migration, like the formation of focal adhesion sites and the reorganization of the cytoskeleton.

4 Models for adhesion kinetics

The development of models for bond formation and rupture is a prerequisite to understand cell–cell interactions at rest or under flow conditions. Bell [8] proposed a theoretical framework for the study of reversible bonds.

4.1 Bell's model [8] and its improvements [37]

The basis of the theory is to consider interactions between a receptor R (located on the cell membrane for example) and a ligand L (on an adhesive plane or on another cell membrane) resulting in the formation of a bond $R-L$. The reversible reaction can be written as:

$$\mathrm{R} + \mathrm{L} \underset{k_d}{\overset{k_f}{\rightleftharpoons}} \mathrm{R\text{-}L}$$

where k_f and k_d are the respective formation and dissociation constants of the reaction. Possible intermediate states may be required, corresponding to positioning (or diffusion) of the receptors and ligands along the membrane so that encounters are possible. These processes may therefore imply a dependence of k_f and k_d on diffusion constants, as a prerequisite for bond association. Such ideas have been used in combination with the geometric constraints to predict the shear threshold effect [20]. In general, we limit ourselves to the only one first–order reaction noted above. The overall constant at equilibrium is defined as $K = \frac{k_f}{k_d}$. Depending on the value of this constant K, the reaction might enhance formation or dissociation of bonds.

We now consider that N_1 receptors and N_2 ligands per unit area react according to the above reaction and that N bonds are formed. Therefore, the time evolution of the bond density N can be written:

$$\frac{dN}{dt} = k_f(N_1 - N)(N_2 - N) - k_d N \tag{1}$$

The long–term equilibrium solution of this equation for N_∞ is given by $N_\infty = \frac{1}{2}(N_1 + N_2 + \frac{1}{K}) - \frac{1}{2}\sqrt{(N_1 + N_2 + \frac{1}{K})^2 - 4N_1N_2}$. Notice that the other solution of the above quadratic equation is not physical [8,56]. It can be shown that the analytical solution of (1) evolves with time to reach N_∞ in steady state. To investigate the dependence of k_d on force F, we suppose that bonds can be broken faster when a larger force is applied, and that their lifetimes will be reduced exponentially with an increase in the applied force F. Therefore we assume :

$$k_d = k_d^0 \, exp\,(\gamma F/kT) \tag{2}$$

where γ is a typical distance, k is the Boltzmann constant, T is temperature. For a given applied force F balanced by N bonds, the steady solution of equation (1), using (2) after replacing F by F/N, gives equilibrium solutions only if F is larger than some typical value F_c necessary to detach the cell. This may be one of the necessary conditions for obtaining cell rolling, in relation to the shear threshold.

Bell also proposed to consider the free energy associated with a single bond, against the distance separating ligands and receptors. The energy landscape is displaced as long as a force is applied, therefore lowering the energy barrier necessary to achieve separation. This idea has been developed further by Evans and Ritchie [37,39] by applying Kramers' theory for reaction

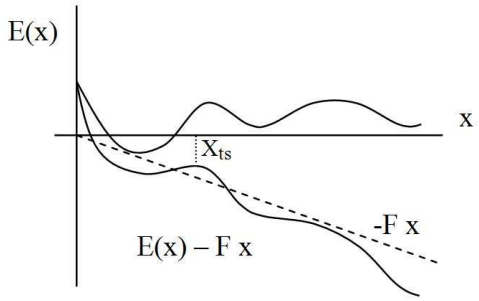

Fig. 5 Typical energy landscape for receptor–ligand bond. Bonds are easier to break under application of a force F. The shape of the well(s) depends on the types of forces and the possible close presence of other bonds. E is the free energy, X is the reaction coordinate. X_{ts} is the position of the transition state under applied force F.

kinetics to bond rupture [65]. By definition, the breakage force is the most probable force that can be obtained during bond breakage tests. Using Monte Carlo simulations [37], they tested the ability of the model to predict peaks in the force at rupture, as a function of the loading rate. First they find a slowly increasing regime of force, in relation with the binding potential. Then a second regime shows a rapid increase independent of the attraction (range of usual experiments such as AFM). Finally, a very fast increasing regime appears corresponding to difficult molecular dynamics simulations. They modelled the results of their simulations in terms of the energy landscape including the application of the force F to the bond (see Figure 5), and obtained a formula for the off–rate frequency ν, which is found to vary like:

$$\nu \sim \nu_0 (\frac{F}{F_\beta})^b \tag{3}$$

where ν_0 is a typical frequency, F_β is a characteristic force, and b is an exponent. Finally the most probable breakage force F^* is found to scale as $F^* \sim ln(r_f)$, where r_f is the loading rate used in the experiments. Such results with three different slopes in the force–rate diagram $F - \nu$ have also been confirmed experimentally [78] in the case of the biotin–avidin complex.

4.2 Adhesion kinetics model [38,31]

The work of Bell was used later by Dembo *et al.* [31] in an approach combining the mechanical aspects of membranes, considered to be elastic, together with the adhesion model described above. The basic idea is to assume that the cell membrane is in close contact with a rigid substrate, when bonds already exist. The membrane is supposed to be pulled by a force T applied at one

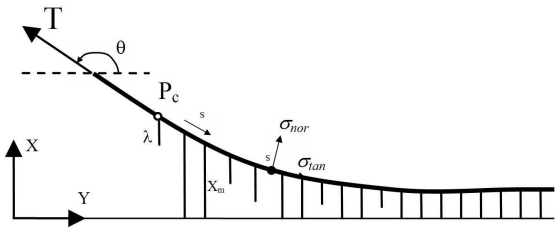

Fig. 6 Sketch of the membrane pulled by force T. P_c is the point where the bond density is vanishing (or is very small). P_c moves in time and is the origin of curvilinear coordinates ($s = 0$). The velocity of P_c is the peeling velocity. The equilibrium of the membrane is written at any point given by its arc length s. Tangential and normal forces σ_{tan} and σ_{nor} (respectively) exerted by bonds are also shown. λ represents the free length of a bond and x_m is the bond length under stress. θ is the angle under which force T is applied. Redrawn from [31].

end, while it is fixed at the other end. A membrane element is assumed to be elastic, and submitted to a tension $T(s)$ and bending moment $M_b(s)$. s is the arc length in the frame moving with the contact point P_c between the membrane and substrate (see Fig. 6).

The equations of motion for the membrane are given by use of beam theory for a thin membrane layer [38,31]:

$$\frac{\partial(T + \frac{1}{2}MC^2)}{\partial s} = -\sigma_{tan} \tag{4}$$

$$M\frac{\partial^2 C}{\partial s^2} - CT = -\sigma_{nor} \tag{5}$$

where C is the curvature, and M is the bending modulus. The contact point P_c is not fixed and can move with a certain velocity. In these equations, the quantities σ_{tan} and σ_{nor} correspond to the tangential and normal forces exerted by the bonds in the normal direction $\sigma_{tan} = N\kappa(x_m - \lambda)\, cos\,\theta$ and $\sigma_{nor} = N\kappa(x_m - \lambda)\, sin\,\theta$, where κ is the elastic bond constant, N the density of bonds as introduced in Sect. 4.1, x_m and λ are respectively the bond lengths after stretch and under zero–load, and finally θ is the angle between the membrane and the substrate. These equations can be solved together with the proper boundary conditions: 1) At the fixed end of the beam (i.e. the elastic membrane sheet), there is no displacement nor any normal stress. 2) At the free end, the applied force and angle are imposed. The density of bonds is assumed to follow equation (1) while the formation and dissociation constants k_f and k_d are given, respectively, by:

$$k_f = k_f^0\, exp(-\frac{\kappa_{ts}(x_m - \lambda)^2}{2kT}) \tag{6}$$

$$k_d = k_d^0\, exp(\frac{(\kappa - \kappa_{ts})(x_m - \lambda)^2}{2kT}) \tag{7}$$

where k_f^0 and k_d^0 are constants, and κ_{ts} is the spring constant of the transition state, so that the only difference between the transition and bonded states is a change in the spring constants used in equation (7), corresponding to different bonds:

- For $\kappa_{ts} > \kappa$, the bonds have a stiffer spring at the transition state therefore $k_d \to 0$ for large x_m. Bonds are mechanically locked: "**catch–bonds**"
- For $\kappa_{ts} = \kappa$ bonds are dissociated easily and are called "**slip–bonds**"

This work resolves the coupling between micromechanical effects (i.e. forces within the bonds) and the macroscopic effects i.e. the global force necessary to achieve "peeling" of the membrane. The main results obtained in the paper of Dembo et al. [31], using mathematical and numerical formulations are the following:

- There is a minimum value of the tension required to move the membrane, which can be regarded as a surface tension like effect as in the case of liquids

- For a constant peeling force, the peeling velocity (motion of point of contact P_c) reaches a steady state after some time

- Experiments conducted for rolling granulocytes [5] are in good agreement with the above model

The assumption of "catch–bonds" suggested in [31] has been a source of interest but it was only after the advent of new methods for measuring small forces such as the Atomic Force Microscope (AFM) [13], the Surface Force Apparatus (SFA) [54] or micropipettes [38] that the existence of catch bonds could be confirmed. Zhu *et al.* [76] combined the use of AFM and flow chamber experiments. Using AFM, they showed that selectin–ligand bonds lifetimes first increased (catch–bond) and then decreased (slip–bonds) as force increases. This was verified for leukocytes subjected to shear stresses. The lifetime increased with increasing shear stress and then decreased, which is a signature of catch bonds.

A further approach [32] based on the above model to study the whole cell deformation will be discussed in Sec. 5.1.

4.3 Models for describing cell rolling – Stochastic models

There have been a number of attempts to model the rolling motion and adherence especially in the case of leukocytes on selectin–coated surfaces. Initial attempts were from Hammer and Apte [46] who proposed to model cells by including microvilli where adhesive springs are located. Then, using statistical fluctuations of receptor–ligand binding, they modelled the whole range of phenomena, including rolling, transient attachment and firm adhesion. To do so, they assumed that the probability P_f for a receptor located at a distance x_m from the substrate to bind the ligand is given by:

$$\frac{dP_f}{dt} = k_f \left(1 - P_f\right) \tag{8}$$

For a single time step $\triangle t$, the solution of (8) is given by:

$$P_f = 1 - exp(-k_f \triangle t) \tag{9}$$

During each time step, the probability P_f is calculated for each microvilli and compared with a random number generated between 0 and 1; if this number is less than P_f, only then a bond is created. Similarly, the probability for a bond to break P_d is given by:

$$P_d = 1 - exp(-k_d \triangle t) \tag{10}$$

The contribution of each bond, as explained previously in Sec. 4.2 is given by a force $F = \kappa(x_m - \lambda)$ in the direction of the bond. Then the contribution of each bond is inserted into the total force and torque exerted on the cell, to which is added the contribution of colloidal forces (van der Waals, electrostatic interactions, steric stabilization forces), hydrodynamic forces and gravity. The velocity field $\mathbf{v}$ is obtained from the mobility tensor $\mathbf{M}$ through:

$$\mathbf{v} = \mathbf{M}\,\mathbf{F} \tag{11}$$

where $\mathbf{F}$ is the total force corresponding to colloidal, hydrodynamic and bond forces. The mobility tensor $\mathbf{M}$ is known [15,44,45] in the case of a sphere travelling through a fluid close to a wall. Finally, the new position of the bonds is calculated from the known velocity field. At each subsequent time interval $\triangle t$, the process is repeated and all variables including the rolling velocity are updated. Simulations [46] provide interesting fluctuating velocities as observed experimentally [56]. Different parameters, such as the receptor density on microvilli, ligand densities, reaction rates, stiffnesses and the hydrodynamic effects, can be obtained and compared with experiments. For example, an increase in the number of microvilli has been shown to reduce the rolling velocities. Satisfactory agreement was found with previous experiments [68].

Such models have been used to predict the influence of shear rate and ligand and receptor densities on the rolling velocity [21]. Another systematic approach suggested by the same group [22] is to represent all results on a state–diagram where different constants, such as the biophysical adhesion properties, are plotted. This mapping allows us to find regimes of transient adhesion, rolling and no adhesion. In a complementary approach [64], simulations of multiparticle adhesive dynamics are produced, including hydrodynamic interactions to explain the effect of collisions to enhance the capture of leukocytes on adhesive walls.

A final more exact model of this type has been introduced recently [20], in order to explain the shear threshold effect. Indeed, when submitted to a slow shear flow, neutrophils roll and the rolling velocity attains a minimum when plotted against the shear rate, which as noted before is known as the shear threshold effect [23]. The recent model [20] uses two supplementary ideas: the first one is to assume an off–rate k_d which enables the description of a catch bond as mentioned earlier [36]. The second idea is to take into account the possiblility of encounters between receptors and ligands through a geometry–dependent probabilistic function, i.e. receptors can only encounter

those ligands being in a close range and only if the interaction time is sufficient. This model has been tested using parameters from the literature and seems to be in fair agreement (base case), but is in even closer connection with experiments when parameters are adjusted (best case). In particular a clear minimum in the rolling velocity is found as a function of the shear rate, thus explaining the threshold effect.

Another approach had been proposed by Zhao and coauthors [103], based on a stochastic model for the micromechanical description of the rolling motion of a cell. The cell's trajectory is considered to be a stepwise function of time with stepsize h and waiting time t_2. The velocity distribution function $p(\nu, t)$ is assumed to obey a Fokker–Planck equation using drift and diffusion coefficients $A(\nu)$ and $B(\nu)$ respectively:

$$\frac{\partial p(\nu,t)}{\partial t} = -\frac{\partial}{\partial \nu} A(\nu) p(\nu,t) + \frac{1}{2}\frac{\partial^2}{\partial \nu^2}[B(\nu) p(\nu,t)] \tag{12}$$

$A(\nu)$ and $B(\nu)$ are estimated by ensemble averaging over the whole cell population. $A(\nu) = (\bar{\nu} - \nu)/\triangle t$, where $\bar{\nu} = \bar{h}/\tau_2$ is the moving average velocity. $\bar{h}$ is the mean of the step size jumps h, τ_2 is the mean waiting time (release of bond clusters) and $\triangle t$ is the observation time for ensemble averaging (time window). They find $B(\nu) = 2\bar{h}/(\triangle t)^2(1+(\sigma_h/\bar{h})^2)\nu$, where σ_h is the variance of h. Analytical expressions can then be obtained for the mean rolling velocity and its variance in the case of homogeneous populations, and separation of temporal fluctuations and heterogeneity can be obtained in other more general cases. Results are in good agreement with experiments [58]. Note that similar approaches based on the concept of master equations [86] have also been developped and tested successfully. To illustrate the fluctuation effects, we present our experiments that were performed in a flow chamber where an endothelial cell monolayer has been cultured. Leukocytes are injected into the stream. As seen in Fig.7, the instantaneous velocity of a rolling leukocyte undergoes fluctuations which we believe are mainly due to irregular bond densities, roughness of the endothelium, and irregular formation and breakup of tethers.

To conclude, we observe that the predictions for the rolling motion of a cell are now reasonably well understood, but there is still a need for introducing the effect of cell deformability, which is discussed in the next Sect. 5.

5 Effect of cell deformability on cellular interactions under flow

In subsection 5.1, we present different ways to model cells at the microscopic or macroscopic levels. Then, in 5.2, we present the cell–interaction models.

5.1 Description of cell models [100]

There has been considerable interest in the mechanical modelling of cells during the past forty years. A cell is a very sophisticated system [100] that

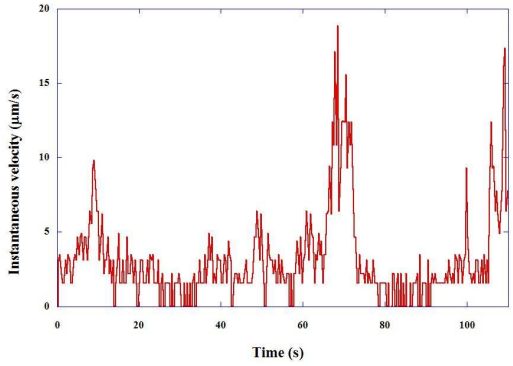

Fig. 7 Instantaneous velocity of a leukocyte rolling on the endothelium in a flow chamber. Fluctuations are clearly visible. Velocities are measured using the time–lapse images taken from a movie of a rolling cell. The flow velocity is around $1\,mm/s$, and the average rolling velocity is estimated to be $3\,\mu m/s$.

cannot be modeled as a continuum although it might be convenient to do so, depending on the scale of the problem considered. Recently, advanced techniques for measuring microrheology have been developed, some of which are quite similar to the ones used by researchers interested in measuring cell–cell adhesion. AFM [1,16], micropipettes [101,51], optical tweezers [13,49], particle tracking microrheology [77,29] have been applied for the investigation of local cellular properties, like the ones involved in cell migration [67].

Cells change their rheological properties continuously. Their cytoplasm exhibits a frequency–dependent behavior, close to that of a physical gel. Its essential properties depend on the organization of filaments, in particular actin. Recent studies [7] on the rheology of model biopolymer networks showed that such systems are highly heterogeneous in space and time and in close relation to biological functions. The cell nucleus can be considered to be elastic or viscoelastic. The membrane has bending properties but elastic properties as well and is extensible like a capsule [6]. Note that this extensibility is more likely due to membrane trafic or unfolding of the membrane. The membrane bending effect, as described by vesicle models with constant area [90], is enough to describe a rich variety of cell motions, in relation with RBCs. "Tank–treading" and "tumbling" motions have indeed been simulated [12] and were previously reported in experiments [89,73]. Describing the cell's intrisic behavior as a function of time and space is still rather difficult to do. Therefore, there have been a few attempts to describe the cell heterogeneity in the literature, one such example is the modelling of cell division based on a non homogeneous cytoskeleton [48].

In order to study cell interactions, cells were first modeled as beads covered by adhesion molecules [22,64]. This is in fact no surprise since leukocytes are usually round and rigid and the main objective was to look at adhesion forces to model such interactions. These studies allowed researchers to construct cell adhesion diagrams and the possibility to model cell interactions. Then other studies considered cell deformation [99,60,32,91] by using nu-

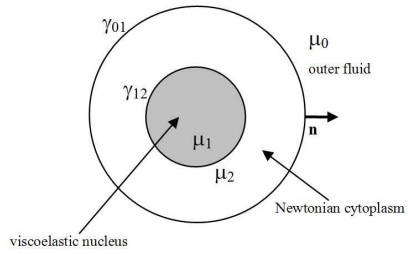

Fig. 8 Sketch of the cell composite droplet. Interfacial tensions γ_{01} and γ_{12} are indicated, as well as viscosities μ_0, μ_1, μ_2.

merical methods designed for tracking moving boundaries. The idea of the composite droplet model for describing a cell with cytoplasm and nucleus was initiated by Tran-Son-Tay's group [60]. They mainly studied the cell rolling behavior in the two–dimensional case and estimated rolling velocities. Another study [32] used a capsule model [6] coupled with Dembo's adhesion kinetics model to look at the influence of flow on adhering cells. They found that an increase of flow rate or a decrease in membrane elasticity both enhance cell deformation, inducing the formation of new bonds, thus increasing adhesion forces, which was verified experimentally.

In the past decades, several new techniques have been developed to study the motion of cells in three dimensions [55,62,56]. Two basic cell models have been investigated: the cell is either a composite droplet made of two viscoelastic fluids [62,56] or is considered as a capsule [55]. In both cases, the adhesion kinetics model [31] was coupled with deformation theories in order to predict how cells interact with an adhesive wall under flow. Major differences were found: in the first model [55], cells took a flat shape with large surface contact area, whereas in the second one [62] cells interacted with the wall by forming only a few tethers at contact and the cell shape became quite elongated, in contrast with the first model [55].

5.2 An example: the viscoelastic cell composite model [56]

In this final section, we consider the problem of a deformable cell in contact with the endothelium, modeled as a planar surface. The cell itself is modeled as a viscoelastic composite droplet, with interfacial tension acting on the cell and nucleus surfaces. Both nucleus and cytoplasm can be considered to be viscoelastic. The adhesive interactions with the wall will be considered in two different ways: either with an attractive–repulsive potential [97], or using the kinetic adhesion model [31] described in subsection 4.2. Let us first describe the problem. A composite droplet as in Figure 8 is considered to have a viscoelastic nucleus with viscosity μ_1 ($100\,Pa.s$) whereas the surrounding cytoplasm has viscosity μ_2 ($35\,Pa.s$), which is smaller than μ_1. The suspending fluid (plasma) has viscosity μ_0 which is much smaller

than the other two viscosities. The equations of motion to be solved are the conservation of mass and momentum equations together with a constitutive law for the viscoelastic fluid. Mass conservation is written as:

$$\bigtriangledown \cdot \mathbf{v} = 0 \tag{13}$$

Conservation of momentum reads:

$$\varrho(\frac{\partial \mathbf{v}}{\partial t} + \mathbf{v}.\bigtriangledown \mathbf{v}) = -\bigtriangledown p + div\,\mathbf{T_t} + \varrho\,\mathbf{g} + \gamma\,C\,\delta(\phi)\,\mathbf{n} + \delta(\phi)\,\mathbf{F_a} \tag{14}$$

where $\mathbf{v}$ is the velocity field, ϱ is the density, p is the pressure field, $\mathbf{g}$ is gravity, C is curvature, $\mathbf{n}$ is the unit normal to the surface of the cell (drop), γ is the interfacial tension acting on the interface, ϕ is the level–set function of the cell boundary (re–initiated to make it a distance function from the interface), δ is the Dirac function, and $\mathbf{F_a}$ is the adhesive force at the wall.

The function ϕ is a solution of the advection equation, and satisfies:

$$\frac{\partial \phi}{\partial t} + \mathbf{v}.\bigtriangledown \phi = \mathbf{0} \tag{15}$$

The total stress $\mathbf{T_t}$ is given as the sum of a Newtonian contribution (due to solvent viscosity μ_s) and an additional stress: $\mathbf{T_t} = 2\mu_s\ \mathbf{D} + \mathbf{T}$, where $\mathbf{D}$ is the symmetric part of the velocity gradient tensor and $\mathbf{T} = \frac{c\,\mu_s}{\tau}\ \mathbf{A}$ is the viscoelastic contribution, $\mathbf{A}$ being the configuration tensor satisfying Maxwell's equation:

$$\frac{\partial \mathbf{A}}{\partial t} + \mathbf{v}.\bigtriangledown \mathbf{A} - \mathbf{A}.\bigtriangledown \mathbf{v} - \bigtriangledown \mathbf{v}^{\mathbf{T}}.\mathbf{A} = \frac{\mathbf{1}}{\tau}(\mathbf{I} - \mathbf{A}) \tag{16}$$

Here τ is the relaxation time of the viscoelastic fluid. The contribution of the polymer viscosity has been introduced here through c which is usually taken as the fractional contribution of the polymer to the zero–viscosity of the fluid μ_s. Therefore, the polymer viscosity is $\mu = c\mu_s$, and the total viscosity of the nucleus is $\mu_1 = \mu + \mu_s = (1 + c)\,\mu_s$. Details can be found in a previous paper [85]. Going back to the adhesive part, two possibilities exist for $\mathbf{F_a}$.

- First case. An adhesion potential $W = w(\frac{d_0}{x})^2[(\frac{d_0}{x})^2 - 2]$ [97] is used and the force is $\mathbf{F_a} = -\partial W/\partial \mathbf{x}$. d_0 is the separation length between the attractive and repulsive regions, w a constant potential and $\mathbf{x}$ is the distance vector from the surface to the point considered (with x being its magnitude).
- Second case. Adhesion kinetics theory. We solve the relationships presented in equations (1-6-7). We use an unstressed length including the presence of microvilli, therefore λ is replaced by the length of the unstressed microvilli and bond in equation (6-7). Then $\mathbf{F_a} = N\kappa(x_m - \lambda)\ \mathbf{n_0}$ as previously shown. $\mathbf{n_0}$ is the unit normal to the adhesion plane.

The variables in the equations are made dimensionless as follows: lengths by R (cell outer radius), velocities by U (maximum velocity of corresponding Poiseuille flow), stresses by $\mu_0 U/R$. The relevant dimensionless numbers in this problem are the Reynolds number $Re = \rho_0 UR/\mu_0$, the capillary number

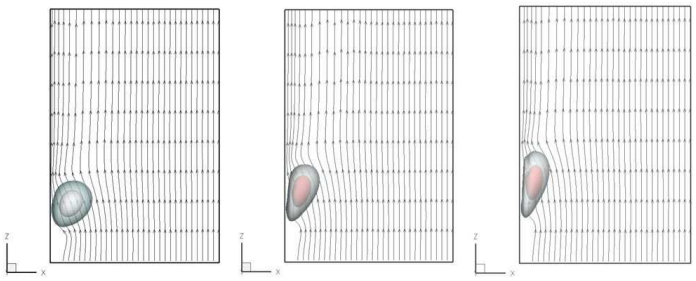

Fig. 9 3D Droplet–wall interaction in the case of an adhesive potential (side view). $Re = 0.5, C_a = 0.167, De = 0.1, Ad = 50, times = 0.82 - 2.46 - 3.94$.

$C_a = U\mu_0/\gamma_{01}$, the Deborah number for viscoelasticity $De = U\tau/R$, the viscosity ratios μ_2/μ_0 and μ_1/μ_0 and the adhesion number $Ad = F_a/\rho_0 U^2$. We will assume that the most two relevant dimensionless numbers are the capillary number C_a and the adhesion number Ad, and keep the other parameters constant. The fluid velocity at the domain walls is assumed to be zero. The level–set function ϕ is reinitialized at each step according to previous procedures [85]. The method used has been presented previously [94,59]. The problem is solved everywhere for $\mathbf{v}$ and ϕ using a code based on the level–set method. A Marchuk–Yanenko operator splitting method is used to decouple the difficulties, i.e. incompressibility, nonlinear convection term, interface motion and viscoelastic problem. A detailed description of the methods used for solving the above subproblems can be found in [43,93] and the details of the numerical code in [92].

Three–dimensional computations of time-dependent cell shapes and streamlines are presented in Figures 9-10. Parameter values for the two cases considered here have been taken from previous studies [31,95,98,68,33,60,87, 62,63,50].

The deformation appears to be an essential factor in modelling of the problem. Several noticeable features are:

- The nucleus is deformed and follows the rest of the cell. It seems to have no further effect, in agreement with previous motility experiments on enucleated cells [35,75].
- Flattening increases the adhesion area below the cell and therefore increases adhesive forces or the number of bonds (Fig.9). This is similar to previous works [32,55,56] but different from another one [62] using the concept of composite drop model.
- The differences are not so marked in these two models. They both describe qualitatively the attraction, with an increased adhesion for the adhesion potential model.
- The rolling velocity from Fig.10 is in the range of a few $\mu m/s$, as observed in our experiments [25,56], and previously shown in Fig.7.

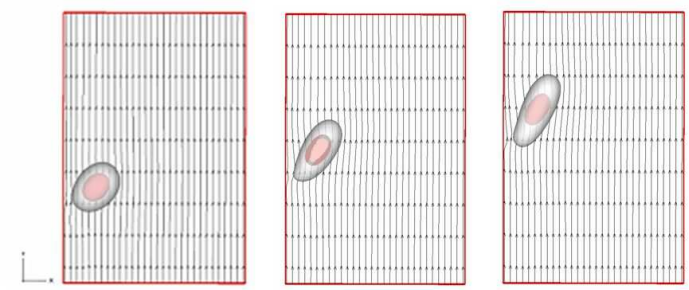

Fig. 10 3D Droplet–wall interaction in the case of the adhesion kinetics model (side view). $Re = 0.5, C_a = 0.167, De = 0.1, Ad = 50, times = 0.6 - 3.0 - 6.0$.

- If the flow rate is large enough, or the adhesion number Ad is sufficiently small, then the cell can be lifted by the flow as already observed in previous works [18].
- Cases with large Ad (Fig.9) lead to cell arrest and a flattened cell adhering to the wall.

6 Conclusions

In this review, we have summarized the important new developments that have taken place in recent years to build a mathematical framework for modelling cell–cell interactions under flow conditions. Using Bell's theory [8] and improvements by Dembo and coauthors [31], we have argued that it is now possible to qualitatively predict and describe the rolling motion of cells on ligand–coated surfaces. Although there have been several interesting computational studies conducted in the recent years, including our own, the problem of cell deformation and interaction under more complex flow conditions is still a major challenge that cannot be resolved without further understanding of the following issues:

- Interactions between cells can be far more complex than those between a cell and a flat wall, which have been considered in this article.

- Cell deformation must take into account the local changes in the rheological and adhesive properties, and not assume the entire cell to be a continuum.

- Cells interact with each other by activating signaling pathways which can induce changes in cell behavior and this should be included in more advanced models.

The above summary of the state–of–the–art should stimulate further interest in this subject, but the new ideas should be first validated in less complex biological systems before they are used to describe the behavior of real cells.

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References

1. A-Hassan, E., Heinz, W.F., Antonik, M.D., D'Costa, N.P., Nageswaran, S., Schoenenberger, C.A., Hoh, J.H.: Relative microelastic mapping of living cells by atomic force microscopy. Biophys. J. **74**(3), 1564–1578 (1998)
2. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., Watson., J.D.: Molecular biology of the cell, third edn. Garland Science (1994)
3. Alon, R., Chen, S., Puri, K.D., Finger, E.B., Springer, T.A.: The kinetics of l-selectin tethers and the mechanics of selectin-mediated rolling. J. Cell Biol. **138**, 1169–1180 (1997)
4. Alon, R., Hammer, D.A., Springer, T.A.: Lifetime of the p-selectin-carbohydrate bond and its response to tensile force in hydrodynamic flow. Nature **374**(6522), 539–542 (1995)
5. Atherton, A., Born, G.V.: Relationship between the velocity of rolling granulocytes and that of the blood flow in venules. J. Physiol. **233**(1), 157–165 (1973)
6. Barthès-Biesel, D., Rallison, J.M.: The time-dependent deformation of a capsule freely suspended in a linear shear flow. J. Fluid mech. **113**, 251–267 (1981)
7. Bausch, A.R., kroy, K.: A bottom-up approach to cell mechanics. Nature Physics **2**, 231–238 (2006)
8. Bell, G.I.: Models for the specific adhesion of cells to cells. Science **200**(4342), 618–627 (1978)
9. Bell, G.I., Dembo, M., Bongrand, P.: Cell adhesion. competition between nonspecific repulsion and specific bonding. Biophys. J. **45**(6), 1051–1064 (1984)
10. Benoit, M., Gabriel, D., Gerisch, G., Gaub, H.E.: Discrete interactions in cell adhesion measured by single-molecule force spectroscopy. Nat. Cell Biol. **2**(6), 313–317 (2000)
11. Bershadsky, A.D., Tint, I.S., Neyfakh, A.A., Vasiliev, J.M.: Focal contacts of normal and rsv-transformed quail cells. hypothesis of the transformation-induced deficient maturation of focal contacts. Exp. Cell Res. **158**(2), 433–444 (1985)
12. Biben, T., Misbah, C.: Tumbling of vesicles under shear flow within an advected-field approach. Phys. Rev. E **67**(3), 031,908 (2003)
13. Binnig, G., Quate, C.F., Gerber, C.: Atomic force microscope. Phys. Rev. Letters **56**(9), 930–933 (1986)
14. Bohnet, S., Ananthakrishnan, R., Mogilner, A., Meister, J.J., Verkhovsky, A.B.: Weak force stalls protrusion at the leading edge of the lamellipodium. Biophys. J. **90**, 1810–1820 (2006)
15. Brenner, H.: The slow viscous motion of a sphere through a fluid toward a plane surface. Chem. Eng. Sci. **16**, 242–251 (1961)
16. Canetta, E., Duperray, A., A.Leyrat, Verdier, C.: Measuring cell viscoelastic properties using a force-spectrometer: influence of protein-cytoplasm interactions. Biorheology **42**(5), 321–333 (2005)
17. Canetta, E., Leyrat, A., Verdier, C.: A physical model for studying adhesion between a living cell and a spherical functionalized substrate. Math. Comp. Modelling **37**, 1121–1129 (2003)
18. Cantat, I., Misbah, C.: Lift force and dynamical unbinding of adhering vesicles under shear flow. Phys. Rev. Letters **83**(4), 880–883 (1999)
19. Cao, J., Donell, B., Deaver, D.R., Lawrence, M.B., Dong, C.: In vitro side-view imaging technique and analysis of human t-leukemic cell adhesion to icam-1 in shear flow. Microvasc. Res. **55**, 124–137 (1998)

20. Caputo, K.E., Lee, D., King, M.R., Hammer, D.A.: Adhesive dynamics simulations of the shear threshold effect for leukocytes. Biophys. J. **92**, 787–797 (2007)
21. Chang, K.C., Hammer, D.A.: Adhesive dynamics simulations of sialyl-lewis(x)/e-selectin-mediated rolling in a cell-free system. Biophys. J. **79**(4), 1891–1902 (2000)
22. Chang, K.C., Tees, D.F.J., Hammer, D.A.: The state diagram for cell adhesion under flow: Leukocyte rolling and firm adhesion. Proc. Natl Acad. Sci. USA **97**(21), 11,262–11,267 (2000)
23. Chen, S., Springer, T.A.: An automatic braking system that stabilizes leukocyte rolling by an increase in selectin bond number with shear. J. Cell Biol. **144**(1), 185–200 (1999)
24. Choquet, D., Felsenfeld, D.P., Sheetz, M.P.: Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages. Cell **88**(1), 39–48 (1997)
25. Chotard-Ghodsnia, R., Drochon, A., Duperray, A., Leyrat, A., Verdier, C.: Static and dynamic interactions between circulating cells and the endothelium in cancer, chap. 9, pp. 243–267. "Cancer modelling and simulation", L. Preziosi Eds. CRC Press (2003)
26. Chotard-Ghodsnia, R., Drochon, A., Grebe, R.: A new flow chamber for the study of shear stress and transmural pressure upon cells adhering to a porous biomaterial. J. Biomech. Eng. **124**, 258–261 (2002)
27. Chotard-Ghodsnia, R., Haddad, O., Leyrat, A., Drochon, A., Verdier, C., Duperray, A.: Morphological analysis of tumor cell/endothelial cell interactions under shear flow. J. Biomechanics **40**(2), 335–344 (2007)
28. Coughlin, M.F., Schmid-Schönbein, G.W.: Pseudopod projection and cell spreading of passive leukocytes in response to fluid shear stress. Biophys. J. **87**, 2035–2042 (2004)
29. Crocker, J.C., Valentine, M.T., Weeks, E.R., Gisler, T., Kaplan, P.D., Yodh, A.G., Weitz, D.A.: Two-point microrheology of inhomogeneous soft materials. Phys. Rev. Letters **85**(4), 888–891 (2000)
30. Décavé, E., Garrivier, D., Bréchet, Y., Fourcade, B., Brückert, F.: Shear flow-induced detachment kinetics of dictyostelium discoideum cells from solid substrate. Biophys. J. **82**(5), 2383–2395 (2002)
31. Dembo, M., Torney, D.C., Saxman, K., Hammer, D.: The reaction-limited kinetics of membrane–to–surface adhesion and detachment. Proc. R. Soc. Lond. B **234**, 55–83 (1988)
32. Dong, C., Cao, J., Struble, E.J., Lipowski, H.H.: Mechanics of leukocyte deformation and adhesion to endothelium in shear flow. Ann. Biomed. Eng. **27**, 298–312 (1999)
33. Drury, J.L., Dembo, M.: Hydrodynamics of micropipette aspiration. Biophys. J. **76**(1), 110–128 (1999)
34. Endlich, N., Endlich, K.: Stretch, tension and adhesion - adaptive mechanisms of the actin cytoskeleton in podocytes. Eur. J. Cell Biol. **85**(3-4), 229–234 (2006)
35. Euteneuer, U., Schliwa, M.: Persistent, directional motility of cells and cytoplasmic fragments in the absence of microtubules. Nature **310**(5972), 58–61 (1984)
36. Evans, E., Leung, A., Heinrich, V., Zhu, C.: Mechanical switching and coupling between two dissociation pathways in a p-selectin adhesion bond. Proc. Natl Acad. Sci. USA **101**(31), 11,281–11,286 (2004)
37. Evans, E., Ritchie, K.: Dynamic strength of molecular adhesion bonds. Biophys. J. **72**(4), 1541–1555 (1997)
38. Evans, E.A.: Detailed mechanics of membrane-membrane adhesion and separation. i. continuum of molecular cross-bridges. Biophys. J. **48**(1), 175–183 (1985)
39. Evans, E.A.: Probing the relation between force–lifetime–and chemistry in single molecular bonds. Ann. Rev. Biophys. Biomol. Struct. **30**, 105–128 (2001)

40. Foty, R.A., Steinberg, M.S.: The differential adhesion hypothesis: a direct evaluation. Dev Biol **278**(1), 255–263 (2005)
41. Friedl, P., Bröcker, E.B.: The biology of cell locomotion within three-dimensional extracellular matrix. Cell Mol. Life Sci. **57**(1), 41–64 (2000)
42. Gardiner, C.W.: Handbook of stochastic methods for physics, chemistry and the natural sciences. Springer–Verlag, Berlin, Germany (1985)
43. Glowinski, R., Pan, T.W., Hesla, T.I., Joseph, D.D.: A distributed lagrange multiplier/fictitious domain method for particulate flows. Int. J. Multiphase Flows **25**, 201–233 (1998)
44. Goldman, A.J., Cox, R.G., Brenner, H.: Slow viscous motion of a sphere parallel to a plane wall. i. motion through a quiescent fluid. Chem. Eng. Sci. **22**, 637–652 (1967)
45. Goldman, A.J., Cox, R.G., Brenner., H.: Slow viscous motion of a sphere parallel to a plane wall: Ii. couette flow. Chem. Eng. Sci. **22**, 653–659 (1967)
46. Hammer, D.A., Apte, S.M.: Simulation of cell rolling and adhesion on surfaces in shear flow: general results and analysis of selectin-mediated neutrophil adhesion. Biophys. J. **63**(1), 35–57 (1992)
47. Hammer, D.A., Lauffenburger, D.A.: A dynamical model for receptor-mediated cell adhesion to surfaces. Biophys J **52**(3), 475–487 (1987)
48. He, X., Dembo, M.: On the mechanics of the first cleavage division of the sea urchin egg. Exp. Cell Res. **233**(2), 252–273 (1997)
49. Hénon, S., Lenormand, G., Richert, A., Gallet, F.: A new determination of the shear modulus of the human erythrocyte membrane using optical tweezers. Biophys. J. **76**(2), 1145–1151 (1999)
50. Herant, M., Heinrich, V., Dembo, M.: Mechanics of neutrophil phagocytosis: behavior of the cortical tension. J. Cell Sci. **118**(9), 1789–1797 (2005)
51. Hochmuth, R.M.: Micropipette aspiration of living cells. J. Biomechanics **33**(1), 15–22 (2000)
52. Hussain, M.A., Merchant, S.N., Mombasawala, L.S., Puniyani, R.R.: A decrease in effective diameter of rat mesenteric venules due to leukocyte margination after a bolus injection of pentoxifylline–digital image analysis of an intravital microscopic observation. Microvasc. Res. **67**(3), 237–244 (2004)
53. Iguchi, K., Oh, G., Ookawa, K., Yanagi, K., Sakai, M., Yamamoto, T., Ishikawa, S., Onizuka, M.: In vivo observation of pulmonary micrometastasis of colon cancer in normal rats. Microvasc. Res. **73**(3), 206–213 (2007)
54. Israelachvili, J.N., Tabor, D.: The measurement of van der waals dispersion forces in the range 1.5 to 130 nm. Proc. Roy. Soc. London A **331**(1584), 19–38 (1972)
55. Jadhav, S., Eggleton, C.D., Konstantopoulos, K.: A 3-d computational model predicts that cell deformation affects selectin-mediated leukocyte rolling. Biophys. J. **88**, 96–104 (2005)
56. Jin, Q., Verdier, C., Singh, P., Aubry, N., Chotard-Ghodsnia, R., Duperray, A.: Migration and deformation of leukocytes in pressure driven flows. Mech. Res. Commun. **34**, 411–422 (2007)
57. Johnson-Léger, C., Aurrand-Lions, M., Imhof, B.A.: The parting of the endothelium: miracle, or simply a junctional affair? J. Cell Sci. **113**(6), 921–933 (2000)
58. Jones, D.A., Abbassi, O., McIntire, L.V., McEver, R.P., Smith, C.W.: P-selectin mediates neutrophil rolling on histamine-stimulated endothelial cells. Biophys. J. **65**(4), 1560–1569 (1993)
59. Kadaksham, J., Singh, P., Aubry, N.: Dynamics of pressure driven flows of electrorheological suspensions subjected to spatially non-uniform electric fields. J. Fluids Eng. **126**, 170–179 (2004)
60. Kan, H.C., Udaykumar, H.S., Shyy, W., Tran-Son-Tay, R.: Hydrodynamics of a compound drop with application to leukocyte modeling. Phys. Fluids **10**(4), 760–774 (1998)
61. Kaplanski, G., Farnarier, C., Tissot, O., Pierres, A., Benoliel, A.M., Alessi, M.C., Kaplanski, S., Bongrand, P.: Granulocyte-endothelium initial adhesion. analysis of transient binding events mediated by e–selectin in a laminar shear flow. Biophys. J. **64**(6), 1922–1933 (1993)

62. Khismatullin, D.B., Truskey, G.A.: A 3d numerical study of the effect of channel height on leukocyte deformation and adhesion in parallel-plate flow chambers. Microvas. Res. **68**, 188–202 (2004)
63. Khismatullin, D.B., Truskey, G.A.: Three-dimensional numerical simulation of a receptor-mediated leukocyte adhesion to surface: Effects of cell deformability and viscoelasticity. Phys. Fluids **17**(3), 53–73 (2005)
64. King, M.R., Hammer, D.A.: Multiparticle adhesive dynamics: Hydrodynamic recruitment of rolling leukocytes. Proc. Natl Acad. Sci. USA **98**(26), 14,919–14,924 (2001)
65. Kramers, H.A.: Brownian motion in a field of force and the diffusion model of chemical reactions. Physica **7**, 284–304 (1940)
66. Lac, E., Morel, A., Barthès-Biesel, D.: Hydrodynamic interaction between two identical capsules in simple shear flow. J. Fluid Mech. **573**, 149–169 (2007)
67. Laurent, V.M., Kasas, S., Yersin, A., Schäffer, T.E., Catsicas, S., Dietler, G., Verkhovsky, A.B., Meister, J.J.: Gradient of rigidity in the lamellipodia of migrating cells revealed by atomic force microscopy. Biophys. J. **89**(1), 667–675 (2005)
68. Lawrence, M.B., Springer, T.A.: Leukocytes roll on a selectin at physiological flow rates: Distinction from and prerequisite for adhesion through lntegrins. Cell **65**, 859–873 (1991)
69. Leckband, D.: Measuring the forces that control protein interactions. Ann. Rev. Biophys. Biomol. Struct. **29**, 1–26 (2000)
70. Lekka, M., Laidler, P., Gil, D., Lekki, J., Stachura, Z., Hrynkiewicz, A.Z.: Elasticity of normal and cancerous human bladder cells studied by scanning force microscopy. Eur. Biophys. J. **28**(4), 312–316 (1999)
71. Leyrat, A., Duperray, A., Verdier, C.: Adhesion mechanisms in cancer metastasis, chap. 8, pp. 221–242. "Cancer modelling and simulation", L. Preziosi Eds. CRC Press (2003)
72. Lu, H., Koo, L.Y., Wang, W.M., Lauffenburger, D.A., Griffith, L.G., Jensen, K.F.: Microfluidic shear devices for quantitative analysis of cell adhesion. Anal. Chem. **76**(18), 5257–5264 (2004)
73. Mader, M.A., Vitkova, V., Abkarian, M., Viallat, A., Podgorski, T.: Dynamics of viscous vesicles in shear flow. Eur Phys J E Soft Matter **19**(4), 389–397 (2006)
74. Makino, A., Glogauer, M., Bokoch, G.M., Chien, S., Schmid-Schönbein, G.W.: Control of neutrophil pseudopods by fluid shear: role of rho family gtpases. Am. J. Physiol. Cell Physiol. **288**, 863–871 (2005)
75. Malawista, S.E., Chevance, A.D.B.: The cytokineplast: purified, stable, and functional motile machinery from human blood polymorphonuclear leukocytes. J Cell Biol **95**(3), 960–973 (1982)
76. Marshall, B.T., Long, M., Piper, J.W., Yago, T., McEver, R.P., Zhu, C.: Direct observation of catch bonds involving cell-adhesion molecules. Nature **423**(6936), 190–193 (2003)
77. Mason, T.G., Weitz, D.A.: Optical measurements of frequency-dependent linear viscoelastic moduli of complex fluids. Phys. Rev. Letters **74**(7), 1250–1253 (1995)
78. Merkel, R., Nassoy, P., Leung, A., Ritchie, K., Evans, E.: Energy landscapes of receptor-ligand bonds explored with dynamic force spectroscopy. Nature **397**(6714), 50–53 (1999)
79. Milln, J., Hewlett, L., Glyn, M., Toomre, D., Clark, P., Ridley, A.J.: Lymphocyte transcellular migration occurs through recruitment of endothelial icam-1 to caveola- and f-actin-rich domains. Nat. Cell Biol. **8**(2), 113–123 (2006)
80. Moazzam, F., DeLano, F.A., Zweifach, B.W., Schmid-Schönbein, G.W.: The leukocyte response to fluid stress. Proc. Natl Acad. Sci. USA **94**(10), 5338–5343 (1997)
81. Moll, R., Mitze, M., Frixen, U.H., Birchmeier, W.: Differential loss of e-cadherin expression in infiltrating ductal and lobular breast carcinomas. Am. J. Pathol. **143**(6), 1731–1742 (1993)
82. N'Dri, N.A., Shyy, W., Tran-Son-Tay, R.: Computational modeling of cell adhesion and movement using a continuum-kinetics approach. Biophys. J. **85**(4), 2273–2286 (2003)

83. Pearson, M.J., Lipowsky, H.H.: Influence of erythrocyte aggregation on leukocyte margination in postcapillary venules of rat mesentery. Am J Physiol Heart Circ Physiol **279**(4), H1460–H1471 (2000)
84. Pierres, A., Benoliel, A.M., Bongrand, P.: Adhesion molecules and cancer. Rev. Med. Interne **20**(12), 1099–1113 (1999)
85. Pillapakkam, S.B., Singh, P.: A level-set method for computing solutions to viscoelastic two-phase flow. J. Comput. Phys. **174**, 552–578 (2001)
86. Piper, J.W., Swerlick, R.A., Zhu, C.: Determining force dependence of two-dimensional receptor-ligand binding affinity by centrifugation. Biophys J **74**(1), 492–513 (1998)
87. Ramachandran, V., Nollert, M.U., Qiu, H., Liu, W.J., Cummings, R.D., Zhu, C., McEver, R.P.: Tyrosine replacement in p-selectin glycoprotein ligand-1 affects distinct kinetic and mechanical properties of bonds with p- and l-selectin. Proc. Natl Acad. Sci. USA **96**(24), 13,771–13,776 (1999)
88. Rosenbluth, M.J., Lam, W.A., Fletcher, D.A.: Force microscopy of nonadherent cells: a comparison of leukemia cell deformability. Biophys. J. **90**(8), 2994–3003 (2006)
89. Schmid-Schönbein, H., Wells, R.: Fluid drop-like transition of erythrocytes under shear. Science **165**, 288–291 (1969)
90. Seifert, U., Lipowski, R.: Adhesion of vesicles. Phys. Rev. A **42**(8), 4768–4771 (1990)
91. Shyy, W., Francois, M., Udaykumar, H.S., Ndri, N., Tran-Son-Tay, R.: Moving boundaries in micro-scale biofluid dynamics. Appl. Mech. Rev. **54**(5), 405–453 (2001)
92. Singh, P., Joseph, D.D.: Fluid dynamics of floating particles. J. Fluid Mech. **530**, 31–80 (2005)
93. Singh, P., Joseph, D.D., Hesla, T.I., Glowinski, R., Pan, T.W.: Direct numerical simulation of viscoelastic particulate flows. J. Non-Newtonian Fluid Mech. **91**, 165–188 (2000)
94. Singh, P., Leal, L.G.: Finite element simulation of the start-up problem for a viscoelastic problem in an eccentric cylinder geometry using third-order upwind scheme. Theor. Comput. Fluid Dyn. **5**, 107–137 (1993)
95. Springer, T.A.: Adhesion receptors of the immune system. Nature **346**(6283), 425–434 (1990)
96. Springer, T.A.: Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell **76**(2), 301–314 (1994)
97. Sukumaran, S., Seifert, U.: Influence of shear flow on vesicles near a wall: A numerical study. Phys.Rev. E **64**(1), 011,916 (2001)
98. Tran-Son-Tay, R., Needham, D., Yeung, A., Hochmuth, R.M.: Time-dependent recovery of passive neutrophils after large deformation. Biophys. J. **60**(4), 856–866 (1991)
99. Udaykumar, H.S., Kan, H.C., Shyy, W., Tran-Son-Tay, R.: Multiphase dynamics in arbitrary geometries on fixed cartesian grids. J. Comput. Phys. **137**, 366–405 (1997)
100. Verdier, C.: Review. rheological properties of living materials: From cells to tissues. J. Theor. Medicine **5**(2), 67–91 (2003)
101. Yeung, A., Evans, E.: Cortical shell-liquid core model for passive flow of liquid-like spherical cells into micropipets. Biophys. J. **56**(1), 139–149 (1989)
102. Zhang, X., Moy, V.T.: Cooperative adhesion of ligand-receptor bonds. Biophys. Chem. **104**(1), 271–278 (2003)
103. Zhao, Y., Chien, S., Skalak, R.: A stochastic model of leukocyte rolling. Biophys. J. **69**(4), 1309–1320 (1995)