Living cell motility

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The motility of living eukaryotic cells is a complex process driven mainly by polymerization and depolymerization of actin filaments underneath the plasmatic membrane (actin cytoskeleton). However, the exact mechanisms through which cells are able to control and employ ‘actin-generated’ mechanical forces, in order to change shape and move in a well-organized and coordinated way, are not quite established. Here, we summarize the experimental results obtained by our research group during recent years in studying the motion of living cells, such as macrophages and erythrocytes. By using our recently developed defocusing microscopy technique, which allows quantitative analysis of membrane surface dynamics of living cells using a simple bright-field optical microscope, we were able to analyse morphological and dynamical parameters of membrane ruffles and small membrane fluctuations, study the process of phagocytosis and also measure values for cell refractive index, membrane bending modulus and cell viscosity. Although many questions still remain unanswered, our data seem to corroborate some aspects of recent physical models of cell membranes and motility.

Keywords: membrane ruffles; small membrane fluctuations; phagocytosis; membrane bending modulus; cell viscosity; defocusing microscopy

1. Introduction

The crawling motion of living cells is a fascinating and intriguing phenomenon that has probably attracted human attention since the invention of the optical microscope in the seventeenth century. Nevertheless, it was only during the last three decades that the internal ongoing mechanisms behind cellular motions began to be understood. Nowadays, the main driving force for cellular motion has been reasonably well established as the resulting effect of the continuous polymerization/depolymerization of the actin filaments network lying underneath the plasmatic membrane. To maintain tight control over the actin polymerization/depolymerization cycle, cells rely on several accessory proteins, which signal actin filaments not only where or when they must grow or shrink but also how fast they must do it (Pollard et al. 2000; Small et al. 2002; Pollard & Borisy 2003). Knowledge of the complete actin biochemistry within the cell, however, is not enough to explain cellular motility from the physical point of view, where all change in motion must come from applied forces. A possible and

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widely accepted explanation of how chemical bond energy from actin polymerization
is transduced into directed cell motion is the Brownian ratchet mechanism, proposed
by Oster and co-workers (Peskin et al. 1993; Mogilner & Oster 1996a, b, 2003), where
polymerization acts as a rectifier for the Brownian motion of the membrane, thus
generating net movement in a given direction. This model does not explain, however,
the cooperative mechanisms leading to the formation of ruffles and lamellipodia,
which probably originate from instabilities, as proposed by Gov & Gopinathan
(2006). These large-scale membrane motions are important for cell movement,
phagocytosis and macropinocytosis (Swanson & Baer 1995; Swanson & Watts 1995;
Small et al. 2002; Pollard & Borisy 2003). In this paper, we summarize the
experimental results obtained by our research group during recent years in studying
the motion of living macrophages and erythrocytes and discuss how these data give
support to physical models of cell membranes and motility, especially the Brownian
ratchet-based models.

This paper is organized as follows. In §2, we make a brief presentation of the
defocusing microscopy (DM) technique, developed by our group, used to generate
quantitative data on the movements of the plasmatic membrane of living cells.
Section 3 is devoted to our results in characterizing both the morphological and the
dynamical parameters of membrane surface fluctuations on macrophages. In §4, the
phagocytosis of parasites by macrophages is analysed. The results from the study of
erthrocytes are presented in §5. Our final concluding remarks are presented in §6.

2. Defocusing microscopy

Transparent objects (phase objects), like the plasmatic membrane of cells, are
not visible in standard bright-field optical microscope. For this reason,
visualization of such objects usually requires the use of more complex optical
techniques, like phase contrast microscopy, differential interference contrast
microscopy or fluorescence microscopy. However, if a phase object sample is
placed under a simple bright-field optical microscope and slightly moved out of
focus, a defocused image appears. Quantitative analysis of the defocused images
can be made through the use of the DM technique (Agero et al. 2003, 2004).

According to DM theory, the contrast patterns forming the defocused image of
a phase object are given by

\[ C(x, y) = \frac{I - I_0}{I_0} = \Delta n [\Delta f - h(x, y)] \nabla^2 h(x, y), \tag{2.1} \]

where \( I \) and \( I_0 \) represent the luminous intensity at \((x, y)\) and the background
luminous intensity, respectively; \( \Delta n \) is the difference between the refractive
indices of the phase object and the surrounding medium; \( \Delta f \) (defocusing distance)
is the position of the focal plane of the objective; and \( h(x, y) \) is the height of the
imaged object, both relative to the bottom of the sample.

If we take \( \nabla^2 h(x, y) \) as an approximation for the local curvature \( \kappa \) at the
surface of the imaged object, equation (2.1) can be used to translate the image
contrast patterns observed in defocused images into surface curvature profiles of
the imaged object. Therefore, DM can be regarded as a method well suited for
studying curvature and curvature changes on a surface. This method can be used
to resolve surface irregularities down to the nanometre scale, depending on the
contrast resolution of the optical system (Coelho Neto et al. 2006).
Macrophages are cells from the innate immune system of vertebrates whose primary function is the phagocytosis of undesirable objects found inside the organism. We studied the motion behaviour of this kind of cell while adhered to microscope cover glasses. Two kinds of membrane fluctuations were detected and quantified: large propagating structures, usually referred to as membrane ruffles, and small random membrane fluctuations (SRMF), which permeate the whole cell surface. A typical image from a spread adhered macrophage where both kinds of fluctuations can be seen is shown in figure 1.

Ruffles can be described as the result of a significant increase in the curvature of the membrane, caused by a burst of actin polymerization concentrated in a relatively small region. These instabilities in the actin cycle can propagate from one region to another before dissipating, causing the ruffles to move over the membrane. As sites of increased curvature, ruffles are easily visualized with DM, appearing as regions of high contrast (figure 1a). From the analysis of the contrast curvature profiles generated by this type of membrane fluctuation, we were able to obtain quantitative data on some of their morphological and dynamical characteristics, such as size and propagation speed (Coelho Neto et al. 2005). These results are summarized in table 1. By following the changes in the contrast profile of these structures as a function of the defocusing distance $\Delta f$, we were able to measure the refractive index of the macrophages, obtaining $n=1.384\pm0.015$ (Coelho Neto et al. 2006).

Table 1. Macrophage ruffle characteristics at 37°C.

<table>
<thead>
<tr>
<th>height (µm)</th>
<th>width (µm)</th>
<th>length (µm)</th>
<th>speed (µm min$^{-1}$)</th>
<th>frequency (ruffles min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5±0.2</td>
<td>0.4±0.1</td>
<td>13±4</td>
<td>6±2</td>
<td>0.5±0.2</td>
</tr>
</tbody>
</table>

3. Membrane fluctuations on macrophages

Macrophages are cells from the innate immune system of vertebrates whose primary function is the phagocytosis of undesirable objects found inside the organism. We studied the motion behaviour of this kind of cell while adhered to microscope cover glasses. Two kinds of membrane fluctuations were detected and quantified: large propagating structures, usually referred to as membrane ruffles, and small random membrane fluctuations (SRMF), which permeate the whole cell surface. A typical image from a spread adhered macrophage where both kinds of fluctuations can be seen is shown in figure 1.

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Contrast produced by the SRMF is significantly lower than that generated by ruffles, as illustrated in figure 1b. Although their level of contrast is not high enough to allow their characteristics to be obtained directly from contrast profiles, quantitative data on this kind of membrane fluctuation were obtained from temporal and spatial autocorrelation functions, calculated pixel by pixel, from the defocused images. An example of both temporal and spatial autocorrelation functions for the curvature over the surface of a macrophage is shown in figure 2. Using this approach, we were able to measure decay time $t$, correlation length $\xi$, root-mean-squared curvature $\kappa$ and amplitude $a_0$ for these fluctuations (Coelho Neto et al. 2005, 2006). These results are summarized in table 2.

The behaviour of ruffles and SRMF were also studied at different temperatures. Analysis of the data obtained provided evidence of actin polymerization involvement in both processes. Confinement of the SRMF to extensions of approximately 0.23 µm, reported to occur on several other types of cells as well (Krol et al. 1990; Levin & Korenstein 1991; Mittelman et al. 1991), supported the model of membrane compartmentalization proposed by Fujiwara et al. (2002), explaining why only a single correlation length and a single decay time were detected in our correlation data. The fact that the measured amplitude $a_0$ of the SRMF reproduced virtually the same step size of a polymerizing actin filament suggested the existence of a close relationship between small fluctuations of the membrane and actin polymerization, which is the central point of Brownian ratchet models. Based on the assumption of ratchet models
that the amplitude of small fluctuations is controlled by the thermal motion of
the free portions of the membrane and on the compartmentalization hypothesis,
and considering the thermal and elastic properties of lipid bilayers (Helfrich
1973), we can write that

\[
\frac{k_B T}{2} = \frac{K_c}{2} \langle \kappa^2 \rangle \xi^2,
\]

where \( k_B \) is the Boltzmann constant; \( T \) is the absolute temperature; \( K_c \) is the
membrane bending modulus; \( \langle \kappa^2 \rangle \) is the mean-square curvature; and \( \xi \) is the
correlation length of the fluctuations. Using the data from table 2, we obtained
\( K_c \approx 3.2 \times 10^{-10} \) J, a value for the bending modulus typical of free membranes,
indicating the consistency of the present analysis (Coelho Neto et al. 2006).

4. Phagocytosis of parasites by macrophages

Another type of experiment on the motility of macrophages was the induction
and analysis of phagocytosis events (Coelho Neto et al. 2005). By using optical
tweezers, we were able to capture single parasites (Leishmania amazonensis, in
amastigote stage) and place them, one at a time, in contact with a selected
macrophage in order to provoke phagocytosis of the parasite. During the
experiments, ruffling activity on the membrane of the macrophage near the point
of contact was continuously monitored before, during and after phagocytosis of
the parasite.

Phagocytosis of the leishmanias occurred in two forms (figure 3). In one form,
which corresponds to approximately 80% of the events, once the ingestion of the
parasite has started, ruffling activity was significantly increased not only in the
region closely adjacent to the forming phagosome but also in a larger area of
membrane surrounding it. The increased ruffling activity at these regions usually
persisted for a while even after the parasite had been engulfed, returning to the
levels observed before the macrophage had contact with the leishmania after one
or two more minutes (figure 4a). In the other form, the engulfment of the parasite
occurs with no visible signs of ruffling activity outside the immediate vicinity of
the forming phagosome. Ruffling activity in the surrounding regions remained
unchanged before, during and after the phagocytosis event (figure 4c).

We observed that phagocytosis occurring in regions where there was no
increase in ruffling activity took approximately twice as long as that occurring in
regions showing increased ruffling activity during the event, suggesting that these
structures may act as a catalyst for the phagocytosis process.

5. Membrane fluctuations on erythrocytes

In order to explore and test the capabilities of DM, we extended the range of
experiments to include erythrocytes, commonly referred to as red blood cells
(RBCs), because they are more complex phase objects, and due to the large
amount of data that has been accumulated on their shape, the fluctuation surface
spectrum caused by the flicker phenomenon and their optical and mechanical
properties (Evans 1983; Strey et al. 1995).
Applying equation (2.1) to a standard discocyte shape RBC and exploring both circular and reflection symmetries, the image contrast produced is given by

\[ C(r) = 2\Delta n \left[ \Delta f - \frac{h_2(r) + h_1(r)}{2} \right] \nabla^2 h_2(r), \]  

(5.1)

where \( h_1(r) \) and \( h_2(r) \) represent the height of the upper and lower surfaces of the cell (Mesquita et al. 2006). It is important to note that \( h_1(r) + h_2(r) \) has a constant value that corresponds to the height of the thickest part of the RBC. A typical image of a defocused RBC and the corresponding contrast/curvature along the horizontal diameter is shown in figure 5.

By numerically integrating twice the data of figure 5b, as described in Mesquita et al. (2006), we obtained the RBC shape profile shown in figure 6.

Thermal motion causes fluctuations in the average thickness of RBC membranes (Brochard & Lennon 1975). Consequently, it also causes fluctuations of RBC curvatures and flickering of image contrast. From these contrast fluctuations, we obtained the RBC curvature spatial and temporal correlation.

Figure 3. Phagocytosis of the leishmanias occurring in two forms. (a–c) Phagocytosis showing increased ruffling activity (approximately 80% of events): (a) Before adhesion of the parasite, the membrane of the macrophage showed low (normal) levels of ruffling activity. (b) After adhesion of the parasite, ruffling activity increased significantly over a large portion of the membrane. (c) Approximately 2 min after the ingestion of the parasite was complete, ruffling activity over the membrane returned to normal levels. (d–f) Phagocytosis showing constant (low) levels of ruffling activity (approximately 20% of events): (d) Before adhesion of the parasite, the membrane of the macrophage showed low (normal) levels of ruffling activity. (e) After adhesion of the parasite, ruffling activity outside the immediate vicinity of the forming phagosome remained unchanged. (f) After the ingestion of the parasite was complete, the level of ruffling activity was still unaltered. The arrows indicate the position of the parasite.
functions, as shown in figure 7. We adapted Brochard & Lennon’s (1975) theory to be able to calculate spatial and temporal correlation functions for the curvature and fit the experimental correlation curves.

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From the average contrast, we were able to obtain the shape profile and the difference between the index of refraction of the RBC and the medium, $\Delta n = 0.042 \pm 0.005$. From contrast fluctuations, we were able to measure the bending modulus of the RBC, $K_c = 2.2 \times 10^{-19}$ J.

6. Concluding remarks

In this work, we summarized the results obtained from the use of DM to study the motility of living cells. Quantitative data on the amplitude and dynamics of surface cell fluctuations allowed us to obtain the optical and mechanical properties of the membrane and actin cytoskeleton of living macrophages and erythrocytes. Cell motility is a cooperative nonlinear phenomenon, which can
display instabilities and coherent propagating structures. We showed that these propagating structures in macrophages are important for the process of phagocytosis. Only recently a few theoretical models have attempted to picture such a complex phenomenon. Our data partially support results from the present models of motility and provide additional quantitative information that can be used in the search for more realistic models.

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