Study of hepatocyte plasma membrane mechanical properties using optical trapping

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Abstract. In this paper we describe the use of membrane tether formation technique which is widely used to study mechanical properties of plasma membranes. This method was successfully used for the direct measurement of parameters characterizing membranes mechanical properties (static tether tension force and effective membrane viscosity) of human hepatocytes (HepG2 hepatocellular carcinoma line). These results allow using this method in future for diagnostics of the cell membrane, evaluating the influence on the mechanical parameters of various factors, including toxins and drugs.

1. Introduction

Mechanical properties of the cell membrane play an important role in the homeostasis of cell due to the influence on a number of membrane-bound enzymes and receptor systems [1]. Properties of the cell membrane of hepatocytes are closely related to the functioning of this type of cells [2].

Published data indicates that previous studies of hepatocyte membrane mechanics utilized bulk indirect methods as electron paramagnetic resonance and fluorescence depolarization [1, 2]. Only relative changes in membrane fluidity but not the absolute values of membrane mechanical characteristics can be obtained using these methods. Nevertheless, the literature describes relatively wide use of membrane tether formation technique for the study the mechanical properties of various cell lines [3-6] using optical trapping (OT) [7]. Thus we used this method to investigate hepatocytes membrane mechanical properties at the single cell level. The experiments were performed on human cell line HepG2.

The first membrane tether formation experiment using optical trapping was described in [6], where the mechanical properties of the membrane of neuronal growth cone were studied. Later the method described in this paper was used to investigate the properties of various eukaryotic and prokaryotic cells [8, 9].

The goal of our work was to measure parameters characterizing membrane mechanical properties of hepatocytes using membrane tether formation method.
2. Materials and methods

2.1. The experimental setup
Optical set-up was previously described elsewhere [10, 11]. Briefly, the optical trap is created by the 1064 nm infrared laser Spectra-Physics BL-106C, focused by the oil immersion objective with a numerical aperture of 1.46 "Plan-Apochromat" 100x/1.46 Oil DIC (Carl Zeiss). Piezo stage P-561 3DD (PI) is used to move the sample relative to the optical trap. Optical set-up is based on AxioImager.Z1 (Carl Zeiss) microscope. Images are captured using EM-CCD cameras Cascade II 1024 (Photometrics) and iXon3 897 (Andor).

2.2. Cell culture preparation
Cells are grown on 18*18 mm coverslips coated with poly-L-lysine (Sigma). 25 μl of poly-L-lysine (0.01% w/v) water solution is applied to each two coverslips and incubated for 30 min at room temperature. After that, HepG2 culture, grown in 60 mm culture dish (Greiner), is harvested by trypsin addition, transferred to polyl-L-ysine coated coverslips and grown in DMEM medium supplemented with 10% fetal bovine serum (Gibco), 2 mM L-glutamine, and gentamicin at 20 μg/ml at 37 º C in atmosphere of 5% CO₂.

2.3. The course of the experiment
The coverslips with cells are attached to microscope slides using double-sided tape (Scotch), forming a channel of about 10 mm in width and 130 μm in depth between the slide and the coverslip. Next, the channel is filled with polystyrene microspheres (3 μm in diameter, Bangs Laboratories) diluted in DMEM. During the experiment the sample temperature is maintained at 37 º C by objective heating ring.

![Figure 1](image1.png)

**Figure 1.** Schematic of the experiment on the membrane tether formation of cells HepG2: 1 – Microsphere fastening on the cell membrane. 2 - The formation of tether. V₁ – tether elongation speed.

![Figure 2](image2.png)

**Figure 2.** Image of membrane tubular structure (“tether”) in the mode of differential interference contrast (DIC) (marked by a white arrow).

Microsphere is captured by the optical trap and is brought into contact with the cell membrane (see figure 1). Approximately 10 seconds later, the microsphere is moved at a constant speed in direction away from the cell surface using the piezo stage, as a result the membrane tether is formed. During
this process, image recording is being performed using a CCD-camera. After predetermined displacement of stage, motion is stopped, but images are still being recorded (for detection of membrane relaxation processes). Finally, the OT is turned off and camera records microsphere retraction to the cell surface.

At the end of the experiment force calibration is performed using viscous drag force. For this purpose the sample (and water solution inside it) is repeatedly moved in opposite directions relative to trapped microsphere using the piezostage. The microsphere is displaced from the center of the trap to the point where force of viscous friction acting on the microsphere is compensated by the trapping force. Trajectory of microsphere is recorded using a CCD-camera, and the relationship between bead displacement and viscous drag force is fitted with a line to determine the trap stiffness.

2.4. Measured parameters and data processing
During the experiment tether tension force \( f \) is directly measured (by bead displacement from trap focus). It was shown [12] that in some cases (including our) the tension force of the membrane tether does not depend on its length:

\[
  f = f_0 + 2\pi \eta_{\text{eff}} v_t
\]

where \( f_0 \) - stationary tension force of membrane tether; \( v_t \) - the rate of tether formation; \( \eta_{\text{eff}} \) - effective viscosity of membrane.

Sequential measurements of tension force at various speeds provides static tether tension force, that is connected to the membrane tension:

\[
  f_0 = 4\pi R_t (T + \gamma)
\]

where \( T \) - in-plane tension of the membrane; \( \gamma \) - membrane-cytoskeleton adhesion term; \( R_t \) - radius of tether.

The measurement of tether radius \( R_t \) allows to get the membrane tension \((T + \gamma)\). However, radius measuring rather complicates the experiment, so we follow the authors of the earlier works - static tether tension force instead of membrane tension is indicated in our work.

The second parameter is effective membrane viscosity \( \eta_{\text{eff}} \) that consists of three terms:

\[
  \eta_{\text{eff}} = 2\eta_m + \eta_s h^2 \ln \frac{R_0}{R_t} + \eta_s R_t^2 \ln \frac{R_0}{R_t}
\]

where \( \eta_m \) - viscosity, determined by the motion of the membrane as a whole; \( \eta_s \) - viscosity, determined by the relative motion of monolayers; \( \eta_c \) - viscosity due to the shift of the membrane relative to the cytoskeleton; \( h \) - thickness of the membrane; \( R_0 \) - the characteristic radius.

The experimental data were processed using the software package ImageJ [13] (Fiji distribution [14]). Microsphere positions are determined as centers-of-mass of inverted and high-pass filtered bead images.

3. Results and Discussion
Uncoated polystyrene microspheres 3 µm in diameter attached strongly to the cell membrane after 10 seconds and formed membrane tethers when pulled away from the cell surface by the optical trap (figure 2). Membrane tethers appear as lineal low-contrast objects when imaged by the microscope in differential-interference contrast mode. When the bead is released from the trap tether retracts and pulls the bead to the cell surface at up to 30 µm/sec.

During membrane tether extraction first the force acting on the bead grows rapidly until it reaches its peak value which corresponds to the moment of membrane tether formation (figure 3). After that it drops to the intermediate value (called plateau force) and remains roughly constant until the end of tether elongation. For the tether in figure 3 pulled at 1µm/sec peak force is about 60 pN and the
plateau force is about 42 pN. As the elongation speed increases the plateau force increases too. Linear regression of this dependence yields effective membrane viscosity $\eta_{\text{eff}} = 0.27 \pm 0.07$ pN·s/μm and static tether tension force $f_0 = 40\pm3$ pN [11]. Using published data on tether radius (about 200 nm) [12] we can estimate membrane tension $T + \gamma \sim 10$ pN/μm or 0.01 mN/m. Tether radius measurement should give us more precise value of membrane tension.

![Figure 3. Tether tension force as a function of bead displacement from the cell surface when the bead is moved at a constant velocity of 1 μm/sec.](image)

Experiments with actin-disrupting agents, such as cytochalasin D, will allow for the determination of cytoskeleton terms of effective membrane viscosity and membrane tension.

4. Conclusion
Membrane tether extraction is a powerful biophysical method that can be successfully used to study hepatocyte membrane mechanics. It allows for the determination of absolute membrane mechanical parameters values and can be used to assess cell-to-cell variability of these parameters. We are planning to analyze the cytoskeleton contribution to the measured parameters by disrupting F-actin. Also the effect of ethanol and other compounds on hepatocyte membrane mechanics will be tested.

5. References
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