Electrophoretic mobility of a growing cell studied by photonic force microscope

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Living cells have spatially localized charged groups such as nucleus, cell walls, and others that can move in an external electric field providing the cell electrophoretic mobility (EPM). We suggest to monitor the EPM of a single living cell during its growth using optical tweezers combined with a position detector. As an example, we studied the EPM during the yeast growth, and we observed a nonmonotonic behavior of the EPM during the cell cycle, such as that the maximal EPM was observed at the initial stage of the growth, strongly reducing when the cell cycle is near its final stage. © *2010 American Institute of Physics*. [doi:10.1063/1.3519474]

In a biological system, proteins, enzymes, and other molecular structures function with a delicate balance between small mechanical forces, chemical reactions, and electrostatic interaction between different parts of the system. Living cells have several spatially localized charged groups such as the nucleus with a negatively charged DNA, cell walls, and others that together with ions in the cytoplasm can move in an external electric field providing the cell electrophoretic mobility (EPM). The electric charges in live cells interact with or produce electric field, which results in enormous dielectric responses, flexoelectricity, and other related phenomena. The electromagnetic properties of live organisms are of considerable fundamental interest and also hold the potential for numerous practical applications. During the cell growth, one may expect changes in the EPM due to a continuous redistribution of cell organelles together with their size changes. The goal of this study is to show that the EPM of a single growing cell can be monitored during the cell cycle using the photonic force microscope (PFM), i.e., the combination of optical tweezers and a high resolution position detection.[1] A typical PFM setup is comprised of an optical trap that holds a probe due to the radiation forces and a position sensing system. In the usual scheme for biophysical experiments, a polystyrene bead chemically attached to the biomolecule or cell under investigation is optically trapped, and the study of its dynamics permits one to gain insights into the mechanics of the object it is attached with.[2] In our scheme, organelles of the living cell with the refractive index higher than its surrounding medium are used as a probe in a PFM geometry.

As an object of the study, we chose the *Saccharomyces cerevisiae* yeast cell. This is an excellent model organism for research in cellular and molecular biology. Since the basic general biochemical mechanisms are highly conserved among all eukaryotes, the most current understanding of the cell cycle processes such as replication, transcription, translation, and protein sorting originally came from experiments performed on yeast.[3] The majority of genes in yeast is homologous to those in humans; hence, molecular biological tools involving yeast are indispensable for the advancement in fields such as anticancer research.[4] Besides being used in the baking industry, yeasts are also important for the production of industrial chemicals and fuels.[5]

The cell cycle of an individual yeast cell can be divided into four phases: mitosis, G1 (gap), synthesis, and G2 (gap). Nuclear division occurs during mitosis. The period after this but before the initiation of nuclear DNA replication, during which the cell monitors its environment and its own size, is known as the G1 phase. DNA synthesis occurs during the synthesis phase. The period between the completion of nuclear DNA replication and mitosis is termed as the G2 phase. The cell cycle of the cell is regulated primarily at a point in the late G1 when buds form and continue growing until they separate from the mother cell after mitosis. Morphological changes undergone by the cell as it enters the cell cycle can be detected under the microscope.

The cells were grown in yeast extract-peptone-glucose (YPD, Broth, Sigma Aldrich, Spain) medium with complete supplement under standard conditions. In all measurements, the cells were placed in home-made microfluidic chamber with electrodes and diluted further in YPD such that a single cell could be trapped with no other cells in the surrounding medium. The pH of the medium was 6.5 to ensure cell viability over the growing process.

A 958 nm optical beam focused by a 100×, NA=1.3 microscope objective was used for the cell trapping. From our previous experiments,[6,7] we know that the optical power at the sample should be less than several milliwatts to permit the cell to grow even though being in the optical trap. In our experiments, with the trapping power around 1 mW and without external electric field, the cell grows following its normal cell cycle. Also, we did not observe cell orientation along the optical axis of the trapping beam when the bud appears. The trapping beam size was around 1 μm in diameter, and the size of the cell was about 5 μm. High-refractive index granules of different sizes embedded in the cytoskeleton are trapped by the focused optical beam, and the cell as a whole is immobilized near the beam focus due to the cytoskeleton elasticity.[8] An additional 635 nm optical beam from a low noise laser coaxial with the trapping beam

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was used for the cell position detection. The forward scattered light of this beam was collected by a 40× objective and detected by a position detector. The resulting signals were then transferred through an analog to a digital conversion card then to a computer for analysis.

Using a dc electric voltage of the known polarity, we found that in our experimental condition, the cell moves at the direction of the applied electric field, i.e., the electrophoretic mobility is positive corresponding to the previous observations.[9]

Parameters of the electric field applied to the optically trapped cell were chosen, guided by the following observations. The electric field has to be minimal in order to reduce a possibility of the relative motion of the charged parts of the cell and therefore to perturb less the growing process. Hence, an ac signal is preferable; it also permits one to use more accurate techniques of data acquisition. A limiting factor here is the Brownian motion of the trapped cell that induces an unavoidable noise of the position detection output signal. We used a 13 V ac voltage at frequencies 1 and 10 Hz that correspond to an electric field around 1000 V/m near the trapping beam focus for our geometry of the fluid chamber channels and electrodes. By this value, we could measure the ac component of the position signal at the different stages of the cell growth. However, we noticed that the cell growth was impossible even when the minimal possible ac electric field was applied continuously. An explanation of this experimental fact from the biochemical point of view needs a special study. In this work, we overcame this difficulty by using the following protocol. A single yeast cell in the beginning of the cell cycle was trapped, and after the position detection alignment, the cell grew without the electric field for 10 min. Then the electric field was switched on for 25 s, and the position detector signal was acquired with an acquisition rate of 2 kHz. After this, the cell grew for 10 min without electric field, and then the EPM measurements for 25 s were performed. To follow the morphological changes of the growing cell, its microscopic images were also taken. With this protocol, we observed the normal cell cycle, and at the same time we had information of the EPM at the specific stage of the cell growth.

Figure 1 shows the signals from the position detector obtained during the acquisition time of 10 s for the different stages of the cell cycle when the frequency of the electric field was 1 Hz, together with the corresponding images of the cell. As seen, the overall motion of the optically trapped part of the cell is periodical; however, during the acquisition period, the amplitude of the oscillation changes up to 20% due to the Brownian motion. In fact, as mentioned above, the optical beam traps the high-refractive parts of the cell that may not spatially coincide with the electrically charged parts moving due to the electric field. Therefore, the Brownian motion may displace the charged parts of the cell relative to the optically trapped area of the cell, causing the changes in the EPM values. All the cells that grew following the normal cell cycle gave results that were completely consistent with Fig. 1. As seen, the highest EPM of the cell is observed when the bud starts to grow at the membrane of the cell [Figs. 1(b) and 1(c)]. As soon as the size of the bud becomes comparable with the size of the mother cell, the EPM decreases [Figs. 1(e) and 1(d)].

In Fig. 2, we summarize our observations for the two frequencies of the electric field. The figure shows how the amplitude of the first harmonic of the periodical motion of the cell changes along the cell cycle. During the first 20 min of the cell growth, the EPM increases about three times relative to its initial value. Then the EPM decreases, and at the end of the cell cycle, it arrives to the same value as in the beginning of the cycle. We can give the following phenomenological explanation of the measurements. When the experiment starts ($t=0$), the mother cell has a certain charge distribution coming from the membrane, proteins, and cell compartments [Fig. 1(a)]. This charge distribution provides the background oscillation amplitude of the optically trapped cell area. When the cell enters in the mitotic phase of the cell cycle that accounts for approximately 10%–20% of the whole cell cycle, the chromosomes in the cell nucleus separate into two identical sets; then the cytoplasm, organelles, and cell membrane divide into two cells. The previous characterization of a yeast cell growth by Raman microspectroscopy combined with optical tweezers[6] showed that during the first 30 min of the cell growth, the Raman peaks corresponding to proteins and lipids increase considerably. Now, we observe that the generation of new cell constituents leads also to an EPM increase (Fig. 2). The EPM decreases after 50 min, when the cell is in the synthesis phase and the nuclei formation in the bud is finished. When the cell moved to the last G2 phase ($t=120$ min), the charge distribution of proteins went back to the initial stage both in the mother cell and bud, showing the complete cell division. The oscillation amplitude has the value similar to those when the cell growth starts. Hence, we can conclude that the cell acquires a strong EPM only during a relatively short period of the mitotic

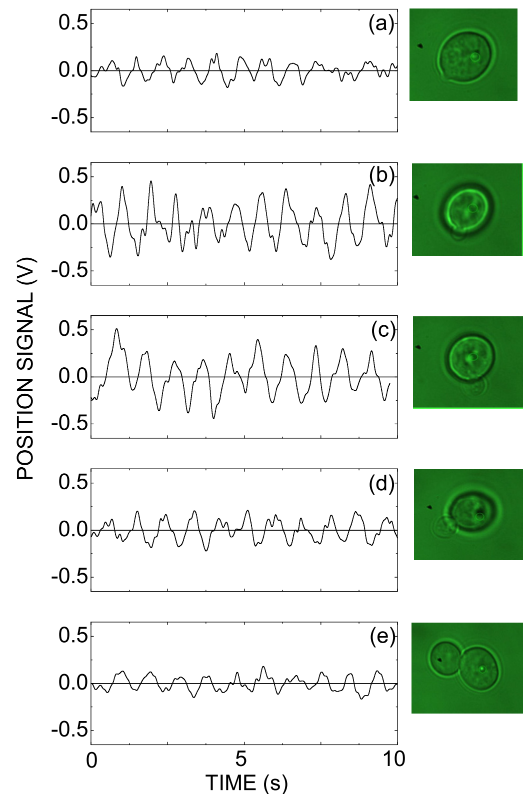

FIG. 1. (Color online) The temporal behavior of the position detector signal during the different stages of the cell growth cycle: (a) start of the experiment, (b) 10, (c) 20, (d) 50, and (e) 120 min. The images near the plots illustrate the morphological state of the cell. The electric field frequency is 1 Hz.

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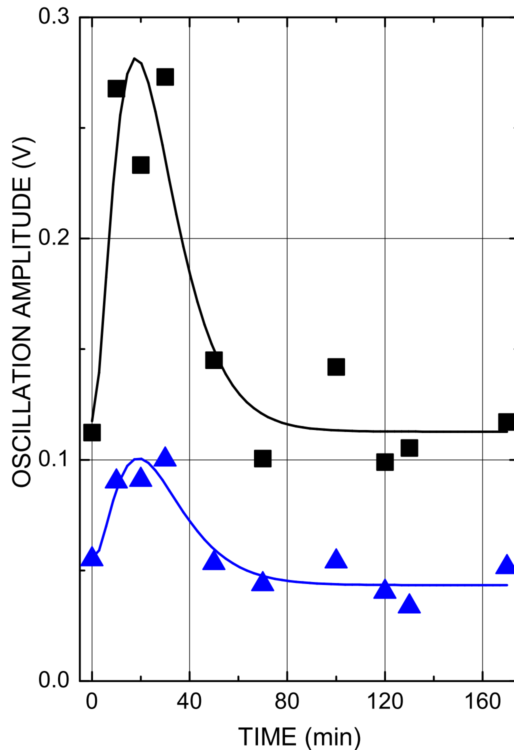

FIG. 2. (Color online) The amplitude of the first harmonic of the cell oscillations during the cell cycle. Two modulation frequencies—1 Hz (squares) and 10 Hz (triangles)—were used. Solid lines were obtained by a fitting of the experimental data to a combination of a polynomial and an exponential function, and they are used only to emphasize the EPM temporal behavior.

phase of the whole cell cycle. In other phases, the cell EPM is much smaller than in the mitotic phase, even though the volume of the cell continues to increase and the processes of the spatial redistribution of the cell components are still active. It is important that the changes in the EPM in the mitotic stage monitored by the photonic force microscopy appear clearly, contrary to the morphology of the growing cell that does not vary considerably.

With ten times higher modulation frequency, we observed smaller amplitudes of the cell oscillation (Fig. 2) due to the viscosity of the cytoskeleton. However, the general behavior was consistent with the measurements at the modulation frequency of 1 Hz.

Future work entails the consideration of all parameters, including a more detailed study of the EPM in different environmental conditions such as changes in the *p*H, hyperosmotic stress, controllable killing of the cell by boiling, or by a chemical protocol. The experimental structure remains suitable for lab-on-a-chip applications. The changes in the EPM may provide additional information by the characterization of the biochemical processes in single living cells.

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