BIOLOGICAL APPLICATION OF ATOMIC FORCE MICROSCOPY USE ON CANCER CELL LINE

Tomankova K, Kolarova H, Bajgar R

Department of Medical Biophysics, Faculty of Medicine, Palacky University in Olomouc, Hnevotinska 3, 775 15 Olomouc, Czech Republic
tomanko@tunw.upol.cz

Atomic Force Microscopy (AFM) has been used to image the morphology of developing tumor cells and their processes. However, it is frequently reported that prior fixation is required for reliable imaging of cells with lower adhesive properties. We used Dry Scanner in Non-contact mode used in biological application of AFM. We imaged the cancer cells before and after photodynamic effect (PDE) and sonodynamic effect (SDE) of photosensitizer $ClAlPcS_2$. PDE was induced by efficient LED source with total light dose of 15 Jcm^{-2}.

We obtained two types of pictures: topography and phase image. In some cases we could observe signs of apoptosis. We also sonicated the cell samples by ultrasonic therapeutic device to improve the effectiveness of PDE. Other results show time-course of ROS production within cells during combination PDE and SDE and their subsequent morphological features and changes investigated by AFM.

The utility of AFM strongly varies depending on the cell type, its membrane structure [1] and adhesion properties [2]. The minimal forces between tip and surface of the sample avoid damage of the biological preparation [3]. Interactions between the cantilever tip and the cell surface are so complex, there is no simple way to control tip-cell interactions and to eliminate the disruptive effect of the scanning cantilever [4]. We used a Dry Scanner and cells were scanned in the Non-contact or the Tapping mode [5]. NC-AFM mode was developed for improving imaging of soft samples by AFM. Difficulties in the proper adjustment of the scanning parameters are often encountered when using tapping–mode atomic force microscopy (TM-AFM) for imaging thick and soft materials, and particularly living cells in aqueous buffer. To increase quality of our images, we scanned cells in non-contact mode (NC-AFM) [6]. Recognition of the cells and control of their surrounding during imaging have already been accepted as essential conditions for cell biological application of AFM [7].

Photodynamic therapy (PDT), originally developed and used mainly as a minimally invasive tumour therapy, has been known for over a hundred years [8]. In clinical PDT, dyes such as porphyries or phthalocyanines are administered to a patient along with irradiation. PDT is predominantly used in anticancer treatment approaches that depend on the retention of photosensitizes in tumour cells and their activation within the tumour through irradiation with light of the appropriate wavelength [9].

The aim of the presented study is to picture tumor-cell surface in air by AFM. We focused to obtain topography pictures and pictures involving elastic properties of cell surface. We examined the cell line G361 before and after induction of PDE. Differences in altitude over surface of cells gave us information about cell damage and about different component parts of the cell wall.

The treatment of the cells with the photosensitizer leads the loss of surface rigidity and eventually to dramatic changes of the cell shape. Individual cells before PDE were characterized by smooth surface without protrusion on the whole surface

References

[1] Méndez-Vilas A., Corbacho I., González-Martín M. L., Nuevo M. J.: Direct surface probing of cell wall-defective mutants of Saccharomyces cerevisiae by atomic force microscopy. Appl. Surf. Sci. **238**, 2004, 51 - 63

[2] Kim H., Arakawa H., Osada T., Ikai A.: Quantification of cell adhesion force with AFM: distribution of vitronectin receptors on a living MC3T3-E1 cell. Ultramicroscopy **97**, 2003, 359 – 363

[3] Dufrêne Y. F., Boonaert Ch. J. P., van der Mei H. C., Busscher H. J., Rouxhet P. G.: Probing molecular interactions and mechanical properties of microbial cell surfaces by atomic force microscopy. Ultramicroscopy **86**, 2001, 113 - 120

[4] You H. X., Lau J. M., Zhang S., Yu L.: Atomic force microscopy imaging of living cells: a preliminary study of the disruptive effect of the cantilever tip on cell morphology. Ultramicroscopy **82**, 2000, 297 - 305

[5] Lehenkari P. P., Charras G. T., Nykänen A., Horton M. A.: Adapting atomic force microscopy for cell biology. Ultramicroscopy **82**, 2000, 289 - 295

[6] Vié V., Giocondi M. C., Lesniewska E., Finot E., Goudonnet J. P., Le Grimellec C.: Tapping-mode atomic force microscopy on intact cells: optimal adjustment of tapping conditions by using the deflection signal. Ultramicroscopy **82,** 2000, 279-288.

[7] Bolshakova A. V., Kiselyova O. I., Filonov A. S., Yu O., Lyubchenko Y. L., Yaminsky I. V.: Comparative studies of bacteria with an atomic force microscopy operating in different modes. Ultramicroscopy **86**, 2001, 121 – 128

[8] Kaestner L.: Evaluation of human erythrocytes as model cells in photodynamic therapy. Gen. Physiol. Biophys. **22**, 2003, 455 - 465

[9] Dougherty T. J.: Photodynamic therapy. J.Photochem. Photobiol. **58**, 1993, 895 - 900

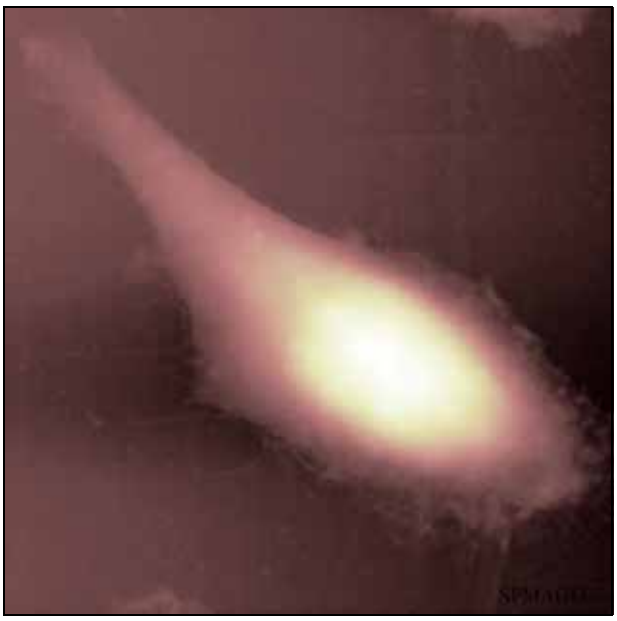

Figure1: One non-irradiated cell of A549 cell line before PDE and SDE. The image was obtained in non-contact topography mode (size 52.33 × 52.33 μm, resolution 300 × 300 pixels, scan rate 50 μms^{-1}). The height of the cell is expressed in colour scale 0 (dark fields) – 2.06 μm (light fields).