DETECTOR DEVELOPMENT FOR NANOMOTION BASED MONITORING OF CELL STATES

Veronika Pfannenstill[a,b], Anne-Céline Kohler[a], Gerhard Schütz[b] and Sandor Kasas[a]

[a]Institut de Physique des Systèmes Biologiques, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

[b]E134 − Institute of Applied Physics − Biophysics at TU Wien

INTRODUCTION

The atomic force microscope (AFM) has provided great new insights in fields like electrochemistry, semiconductor science or molecular biology and has opened the door to a new field, we today call nanotechnology. Moreover, the development and the application of novel technologies resulted in solving important problems. In 2013, Longo *et al*. have taken advantages of the sensitivity of the cantilever to assess the viability of mammalian cells and bacteria with an unprecedented temporal resolution[1]. Further studies demonstrated the use of this technique, called nanomotion detection, to determine the effect of chemicals, such as cytochalasin D, on mammalian cells[2]. Here, we have used this technique to investigate the delivery and effect of antimitotic drugs to cancer cells and established a procedure for fast and reliable detection of the activation of Jurkat cells. The obtained results allow us to make a clear distinction between treated, untreated, activated and dead cells and pave the way towards personalized medicine.

NEW METHODOLOGY

The AFM is standardly used in a configuration that raster scans the cantilever over the “sample” probing its surface. In contrast, we mount the “sample” directly on the cantilever and use it as a sensitive probe for “sample” activity (Figure 1)[2].

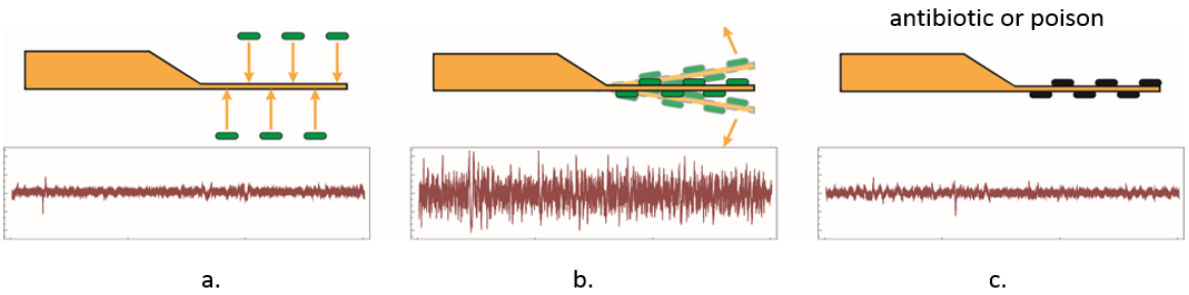

Figure 1: Schematic representation of the methodology used in this work. Upper panels show the principal experimental steps whereas the lower panels depict the cantilever movements as a function of time. a) First the specimen of interest (in green) is attached onto an AFM cantilever with molecules. b) If the specimen is alive or if it undergoes conformational changes, the cantilever oscillates. c) The presence of antibiotics or chemicals in the medium induces death or inhibition of the sample and the cantilever’s oscillations reduce or stop.

Since the movements of the tip can be accurately recorded to detect even individual atoms, it is clear that any movement of any microscopic objects, e.g. cells or bacteria, can be detected if deposited onto the cantilever. Simply, if the organism on the cantilever is alive or undergoes conformational changes it induces oscillations of the cantilever (Figure 1a,b)[1],[3]. Its extreme sensitivity allows to detect motions as small as 0.1 Å. Recently, very first experiments demonstrated that the method can be used to explore numerous types of samples, starting from single proteins to living mammalian cells[3],[4]. It has been already shown that the technique can be used for rapid, in a timeframe of minutes, detection of bacterial susceptibility to antibiotics[1]. Namely, as soon as the

sample is chemically inactivated by fixing agents, antibiotics or poisons, the oscillations of the cantilever decrease (Figure 1c). This information allows us to assess and study the sensitivity of the investigated system to the additionally injected chemical.

RESULTS AND DISCUSSION

We have applied this technique in the fields of immunology, which is, to our knowledge, the first time it has been done. The results obtained, by analysing mechanical oscillations, allow us to identify various cell states, e.g. T cell activation (Figure 2). These experiments were done by adding OKT3, an antibody that induces T cell activation, into the cell medium. Figure 2 shows a clear drop in oscillations upon addition of OKT3 which could be attributed to the spreading and reduction of motion of the cells.

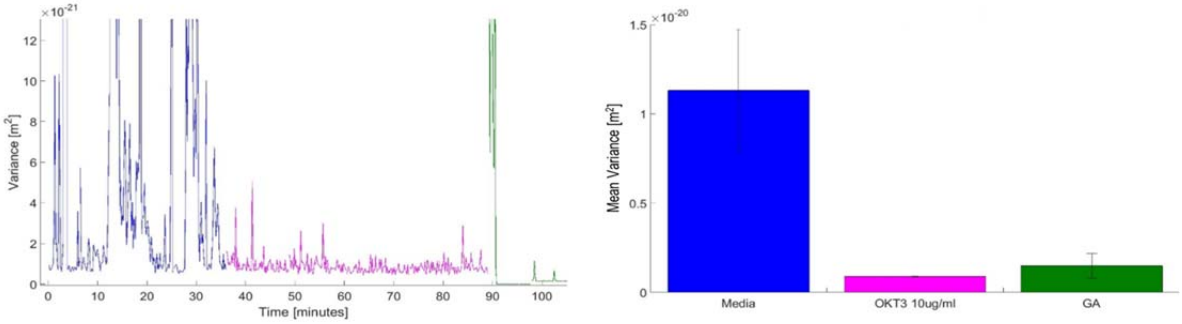

Figure 2: Nanomotion oscillation pattern of Jurkat cells exposed to OKT3 antibody that induces T cell activation. It is possible to clearly differentiate between the three states of the T cells: cells in normal media (blue), activated cells (pink) and dead cells (green).

In contrast, the control experiments with non-activating antibodies did show a constant amplitude of oscillations (data not shown). These results are of particular interest since some new cancer treatments use the patient's immune system and more specifically its T cells to kill cancer cells. A rapid test for T cell activation could therefore potentially lead to important breakthroughs and more efficient anticancer therapies in a time frame of hours/days.

CONCLUSION

It is clear that the presented AFM technique has the potential to revolutionize the fields of drug discovery, medical diagnosis and oncology as demonstrated in the presented tests (Figure 2) above. Further applications could involve sterilization assessment in hospitals and life detection in extreme environments. In addition, the technique may have the potential to serve as a biosensor and therefore change considerably the detection of toxic agents, e.g. environmental pollutants and warfare agents. The results are very encouraging and require subsequent experimental and theoretical work.

ACKNOWLEDGMENTS

This work was supported by a fellowship of the Zeno Karl Schindler Foundation (V.P.), by the Swiss-European Mobility Program (V.P.) and the FWF-Forschungsbeihilfe.

REFERENCES

[1] G. Longo *et al.*, Nat. Nanotechnol., **8** (7), pp. 522–526, 2013.

[2] S. Kasas *et al.*, Proc. Natl. Acad. Sci., **112** (2), pp. 378–381, 2015.

[3] L. Alonso-Sarduy *et al.*, PloS One, **9** (7), p. e103674, 2014.

[4] S. Kasas, P. Stupar, G. Longo, and G. Dietler, Med Sci (Paris), **31** (4), pp. 369–371, 2015.