Quantitative Measurement of Permeabilization in Living Cells by Terahertz Attenuated Total Reflection

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***Abstract*—Using Attenuated Total Reflection (ATR) imaging technique in the terahertz domain, we demonstrate non-invasive, non-staining real time measurements of cytoplasm leakage during permeabilization of live MDCK cells by saponin at low concentration. The origin of the contrast observed between cells and culture medium is addressed by both experimental and theoretical approaches, and demonstrated to give access to permeabilization dynamics for two close saponin concentrations (50 and 75 µg/ml).**

I. Introduction

Reversible permeabilization of live cells is a complex and increasingly addressed issue, whether it is for medical application, or in lab research protocols where a constant effort is made to reach more realistic investigation conditions in biological systems. It is characterized by increased molecules transfers through the cell membrane. Applications cover anticancer drugs or imaging markers deliveries, gene therapy, etc. Besides complex viral vectors that rise potential immune issues, delivery of biomolecules can be done mechanically or chemically. Reversible permeabilization is mostly obtained by techniques creating pores into the membrane, the most commons being electroporation, use of non-ionic detergent and pore-forming toxins [1-2].

The terahertz region has been shown to have potential in biomedical applications, but strong experimental limitations had long kept the study of biological objects down to the single purified molecule, simplified and/or pre-treated biological structures. Recent works demonstrated the possibility to spectroscopically address more complex systems, as cells and even accessible tissues or small organs [3-6].

II. Results

Using Attenuated Total Reflection (ATR) in the terahertz domain, we have been able to follow the permeabilization of a cell layer in physiological condition, without any marker nor sample preparation. The origin of the contrast observed between cells and culture medium is addressed by both experimental and theoretical approaches, and used for following the cytoplasm leakage during permeabilization of MDCK cells by saponin at low concentration.

A 10-µm thick layer of epithelial MDCK cells was grown to confluence on a high resistivity silicon patch, in supplemented culture medium, incubated at 37°C 5% CO_2. Before each experiment, the patch was washed with HBSS and let reach room temperature (21°C) in HBSS + 10 mM HEPES as a buffering solution. The patch was then put on top of the silicon ATR prism [5]. Half of the growing layer was kept, and the other half of the patch was scratched free as a reference S_{ref} for the terahertz signal S originating from the cell layer. A recording visible camera was installed above the setup to make sure no cell detachment occurs, as the cells have to lay in the probing evanescent field (of an approximate 20 µm thickness) occurring at the surface of the patch.

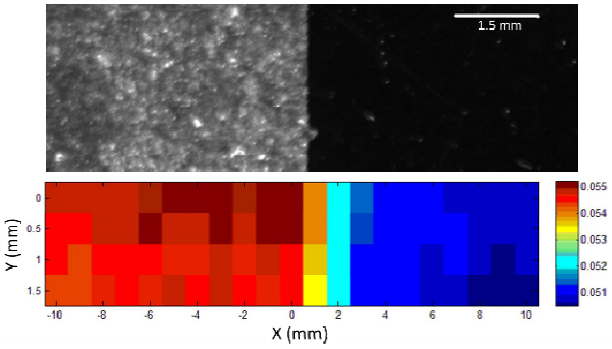

Fig. 1. Optical (top) and terahertz ATR (bottom) images with epithelial cell monolayer (left) compared without cells (right).

Displacement of the patch provides images of the cell layer (Fig. 1), as well as the terahertz contrast defined by :

$$\Delta = \left(S - S_{ref}\right)_t / \left(S - S_{ref}\right)_{t=0}$$

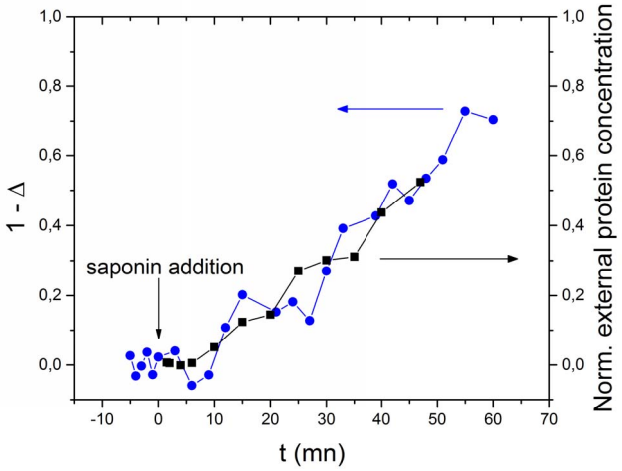

Fig. 2. Normalized extracellular protein concentration (black squares) and normalized THz contrast (1-Δ, blue rounds) after addition of 75 µg/ml of saponin at 21°C, at time t = 0.

At time t = 0, saponin was added at 75 µg/ml. Saponin is a non-ionic detergent that creates reversible non-specific pores in cell membrane at low concentration.

To quantify protein leakage during permeabilization, a BCA assay was performed. This assay is a standard colorimetric assay, determining global protein concentration [7]. In order to stay in the detection range, the assay had to be made on very small extracellular volumes (200 µL), in 24 wells plates. The resulting external normalized protein concentration provides a really good quantitative agreement with inverse terahertz measurements (1-Δ), as shown in Fig. 2. Complementary ionic

permeabilization experiments and theoretical approach underlined a negligible participation of small ionic species and a high expected proteins absorbance. The terahertz contrast is therefore understood as mainly coming from intracellular protein content.

A first example of differential permeabilization dynamics depending on the saponin concentration is show in Fig. 3, for two close concentrations. The comparison between the two curves underlines a first common delay up to 10 mn, then a rapid decrease of Δ in the case of 75 µg/ml to a plateau between 0.9 and 0.8 whereas in the 50 µg/ml case, the delay last around 20 mn before Δ decreasing at a slower rate. Such delays are in agreement with existing studies [2], but had never been reported with this precision level nor with a non-invasive method. These dynamics would be of great interest for whom wants to find a good balance between permeabilization and its reversibility, as protein content lost is linked with viability.

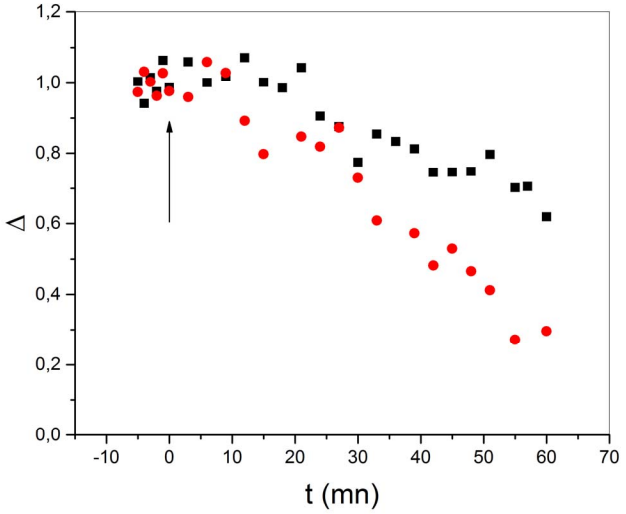

Fig. 3. Δ variation for two different saponin concentrations introduced at t=0 mn (black arrow), at 50 µg/ml (•) and 75 µg/ml (●).

III. SUMMARY

We then demonstrated non-invasive, non-staining real time measurements by terahertz radiation of cytoplasm leakage dynamics during permeabilization of live MDCK cells by saponin at low concentration. Taking advantage of the free space around and above the ATR setup, this technique allows various equipment additions, which is of great interest for further investigation of biological samples.

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