Probing Living Cells by THz Attenuated Total Reflection. Application to Quantitative Permeabilization Measurement

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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 molecule transfer through the cell membrane. Applications cover anticancer drugs or imaging markers delivery, gene therapy, etc. Reversible permeabilization is mostly obtained by techniques creating pores into the membrane, the most commons being electroporation, non-ionic detergents 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].

A 10-µm-thick layer of epithelial MDCK cells was grown on a high resistivity silicon window, and then put on top of a silicon ATR prism [5]. An evanescent field resulting from internal reflection occurs at the surface of the window, probing an approximate thickness of 20 µm in the biological medium. No staining nor any sample preparation are needed in this non-invasive imaging device.

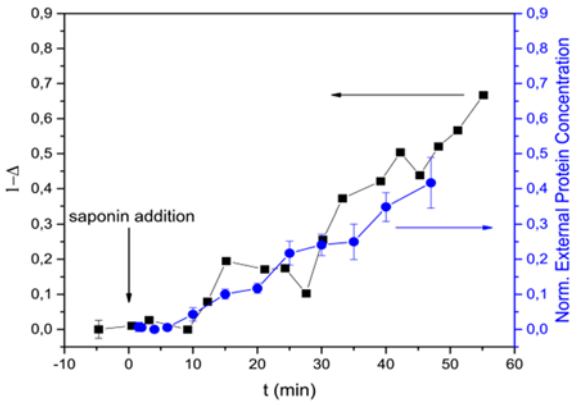

Fig.1. Normalized extracellular protein concentration and normalized THz contrast.

Half of the cell layer was kept untouched, and the other half was scratched free as a reference S_{ref} for the terahertz signal S originating from the cell layer. Displacement of the patch provides images of the cell layer, as well as the terahertz contrast defined by $\Delta = (S - S_{ref})_t / (S - S_{ref})_{t=0}$. At time $t = 0$, saponin (a non-ionic detergent) was added, creating reversible non-specific pores in the cell membrane [2]. We found a very good correlation between the decrease of Δ during permeabilization and the increase of extracellular protein content [7] (using a classical BCA [8]), as shown in Fig.1. A model mixing a broad experimental screening of various protein solutions, fitted by a theoretical approach, has been developed, linking Δ to proteins molecular weight and concentration.

A first example of permeabilization dynamics study is shown in Fig. 2, for two close saponin concentrations. Such dynamics are in agreement with the few existing studies, but had never been reported with this precision level nor with a non-invasive scheme. Comparison with usual permeabilization quantification methods will be discussed.

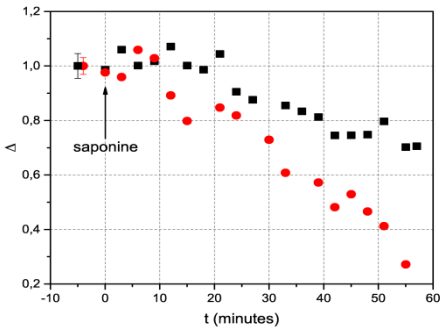

Fig. 2. Δ variation for 2 different saponin concentrations at t=0 min [50 µg/ml (■) and 75 µg/ml (●)].

References

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