Predict Cells Viability, Proliferation and Metabolic Status, Based in One Unique and Simple Assay

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Abstract – A new method to simultaneously predict cells viability, proliferation and metabolic status, in a rapid, simple but also specific and sensitive mode was developed. The method is based on mid-infrared (MIR) spectroscopic analysis of cells. As model system were used Human embryonic kidney (HEK) 293 cells and T-lymphocytes. After submitting cells to different environments as the toxic dimethyl sulfoxide, or metabolic activation, cells viability was analyzed by optical microscopy after coloration with trypan blue, and the cell count was determined with a Neubauer hemocytometer. The principal component analysis (PCA) of the cells second derivative spectra enabled to discriminate the cells viability and the cells proliferation as assayed by conventional methods, while spectra PCA and Hierarchical Cluster Analysis (HCA) enabled to discriminate T-cells metabolic activation. The new methods, based on MIR spectroscopy, present the advantages of being applicable in automatic, simple and high-throughput mode in relation to the conventional methods. _

Keywords – Cells viability, Cells proliferation, FTIR spectroscopy

I. INTRODUCTION

The *in vitro* analysis of cell viability and proliferation are widely applied on diverse biological areas, including e.g., drugs screening of anti-neoplastic compounds, cytotoxic assays and to increase the knowledge of metabolic pathways. The methods currently in use, all present advantages and limitations while complementing each other. For example, diverse viability assays are based on the disruption of the cell membrane, however, a decrease in cell metabolism or even cell death may occur without the immediate disruption of the cell's membrane. The assays that relate the number of cells with their metabolic activity are affected by the cell metabolic status [1,2]. Therefore, to obtain a more realistic view of the cell metabolic status and its mitotic capacity, diverse methods should be used. However, that leads to multiple time consuming or expensive techniques. The present work explored how MIR spectroscopy could be used in a simple procedure enabling to simultaneously predict the cells viability, proliferation, and metabolic status.

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II. MATERIALS AND METHODS

` Human embryonic kidney (HEK) 293 cells grown on DMEM medium supplemented with 10% fetal bovine serum (FBS), and peripheral blood mononuclear cells and T-lymphocytes grown on RPMI with 10% FBS were used.

HEK cells were submitted to the toxic dimethyl sulfoxide (DMSO), while T-lymphocytes were activated by a mitogen.

Conventional cell viability was analyzed by optical microscopy after coloration with trypan blue, and the cells proliferation was predicted by cells count with a Neubauer hemocytometer after 48 h culture.

MIR spectra of cells pellet were acquired as described in Rosa *et al.* [3] and processed as described in Sales *et al.* [4].

III. RESULTS AND DISCUSSION

As expected, DMSO affected cells viability and proliferation (Fig. 1), being observed cells viability between 60 to 90 % and cells proliferation between 1.6 $X10^5$ to $5.9x10^5$, as obtained by conventional methods, i.e., by cells coloration with trypan blue and subsequent observation at the optical microspcope, and the cells count with the Neubauer hemocytometer, respectively.

The principal component analysis (PCA) of the second derivative spectra of the HEK cells, enabled to discriminate either cells viability either the cells proliferation as obtained by conventional methods (Fig. 1).

PCA and HCA of second derivative spectra of lymphocyte T also enabled to discriminate resting T cells (red) and mitogen activated T-cells (blue) (Fig. 2).

The advantage of the new method, is that is conducted in an automatic and high-throughput mode, as the MIR spectra is acquired with samples in a microplate with 96 wells. The method is based on the direct analysis of cells pellet, without the need of further reagents or more manipulations besides a simple step of the sample's dehydration. Therefore, the method based on MIR spectroscopy can represent a simple and economic method enabling to predict the cells viability, proliferation, and metabolic state.

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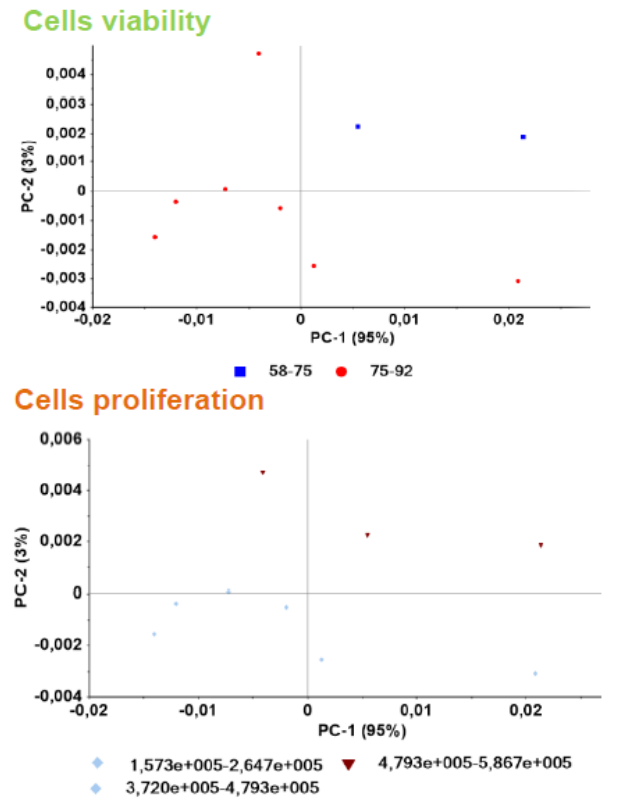

Fig. 1. PCA of second derivative spectra of HEKs cells pellets in function of the cells viability and cells proliferation as analyzed by conventional assays.

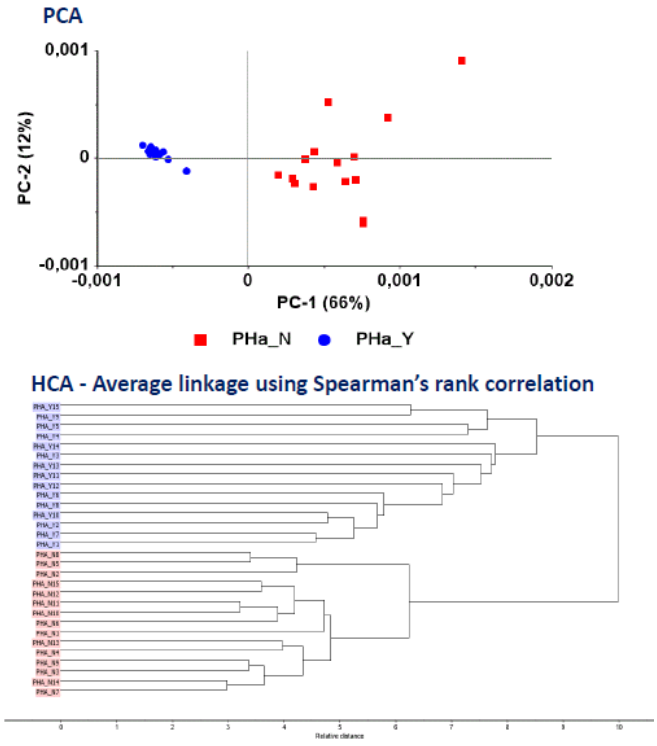

Fig. 2. PCA and HCA of second derivative spectra of lymphocyte T not activated and activated by a mitogen.

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