Morphological surface analysis to study cell viability induced by proliferative and toxic treatments

> " *The use of profilometry in determining changes in cell morphology can be considered an exciting tool for screening potential drugs and toxic materials, distinguishing between the two greatest models of cell death."*

During apoptosis and necrosis processes, dramatic changes in cell volume occur in the early stages of the treatment. Distinguishing between these processes is crucial from a biophysical and therapeutical point of view. Apoptosis is a programmed process of cellular death, while necrosis is an accidental death resulting from environmental perturbations. When a treatment induces apoptosis instead of necrosis, it is more likely to have a better outcome. In addition, cell proliferation processes cause changes in cell volume as a measure of cell growth.

In this study, we show non-invasive morphological surface analysis techniques matching well-assessed biochemical methods to clearly distinguish physiological from pathological cells. Briefly, profilometry analysis allows variations in cell volume induced by suitable nanoparticles (NPs) as precursors

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of proliferation and cell death compatible with cell viability responses measured by conventional methods.

This project is intrinsically interdisciplinary since it requires the characterization of different materials and their interaction with the biological entities of interest, as appreciated on the different backgrounds of the team members. The group led by Dr. Ferrari aims at studying wettability processes, adsorption, and aggregation of amphiphilic molecules at the liquid-solid interface, the preparation of highly liquid repellent coatings, and the characterization using AFM and 3D-profilometry techniques. The group led by Dr. Morán focuses on the development of controlled delivery systems and their in vitro characterization under 2D (bulk and substrate) and 3D conditions.

◼ Measurements

The S neox 3D profilometer was used due to its capability to analyze large surfaces and obtain important surface parameters from the scans. The surface characterization using profilometry was done according to the ISO 25178 standard, which provides the rules for the three-dimensional parametric assessment of surface textures.

The entire surface of the individual coverslips—containing cells under the studied conditions—was analyzed using Confocal mode. Cells in selected areas were chosen, and the corresponding profiles on height (H) and length (L) were analyzed with the SensoSCAN S neox software. The corresponding shape factor (SP) was determined as a function of the H/L values.

The advantages of optical scanning profilometry against atomic force microscopy (AFM) are multiple: non-invasive and non-destructive characterization and the possibility of atunalyzing larger surfaces not being limited to a few cm^2. Moreover, measurement times are reduced compared with demonstrated experience of AFM technique [2, 3], making optical profilometry a microscopic technique which offers more qualitative and quantitative information in terms of high resolution at nano-microscales. Despite no fluorescent protein markers or optically active dyes being required for this technique, 3D scanning profilometry in Confocal and Interferometric modes can provide high accuracy in cell geometry parameters assessment (Fig.1a). Consequently, 3T3 fibroblasts under standard conditions show a typical bipolar or multipolar structure with elongated shapes. In addition, epithelial-like cells as HaCaT, HeLa and A431 cells are polygonal in shape with more regular dimensions, growing in discrete patches. Due to the NPs treatment, 3T3 fibroblasts showed an oval appearance with no filopodia. Epithelial cells undergo a massive lack of boundaries between the cells and shape irregularity.

The analysis of the obtained profiles (Figure 1b) has allowed evaluating, from a quantitative point of view, several structural parameters, such as the height and length of control cells, and those subjected to treatments for the induction of cell proliferation and cell cytotoxicity.
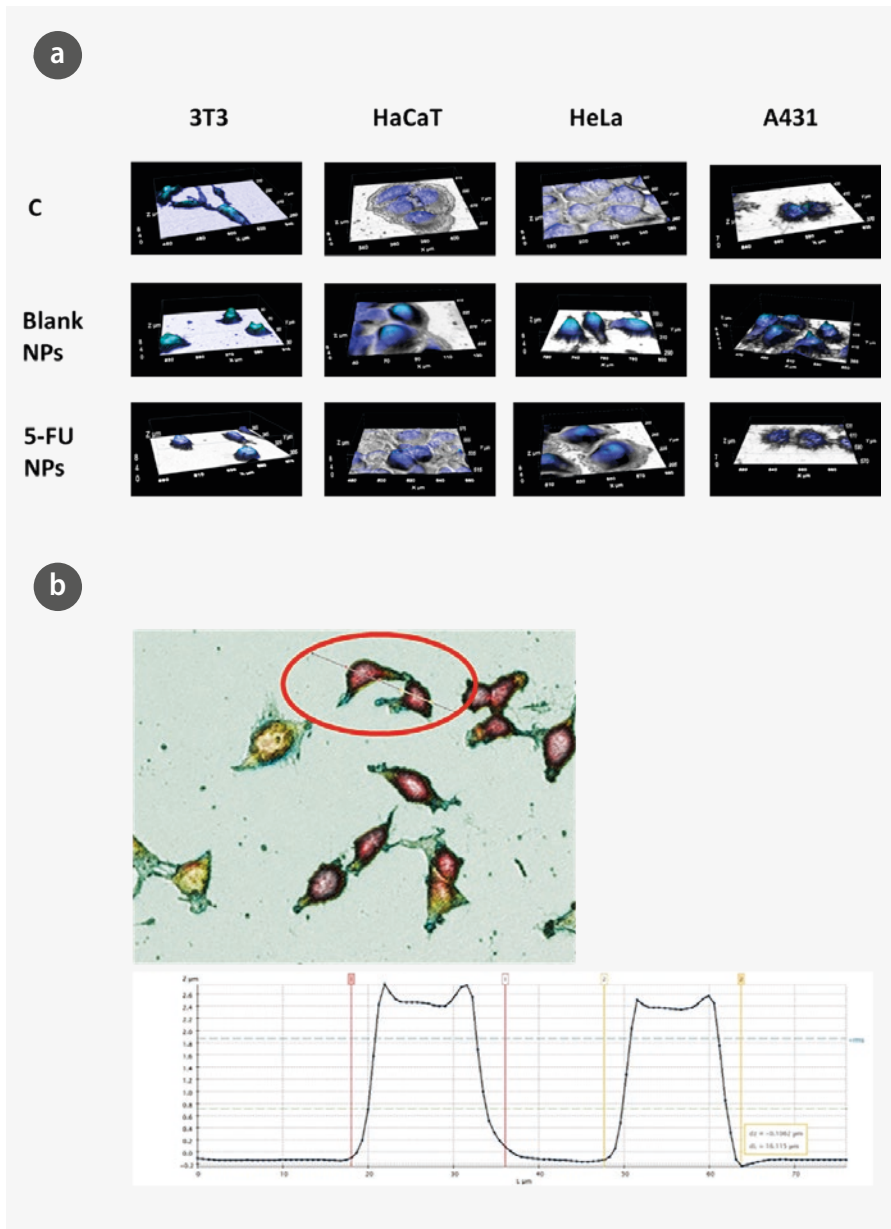
Fig. 1. Representative 3D profilometry images in Confocal mode (magnification 20x) of fibroblasts (3T3 cell line), keratinocytes (HaCaT cell line), epithelial carcinoma (HeLa cell line) and squamous cell carcinoma (A431 cell line) under standard conditions (control cells -C) and after incubation with bare NPs (blank NPs) and 5-FU containing NPs (5-FU NPs) (a) deduced by the corresponding profile sections, of which an example is shown in (b). ("3D profilometry and cell viability studies for drug response screening" by M. C. Morán, F, Cirisano and M. Ferrari is licensed under CCC).

Changes in the morphology of the cells and their shape factor as a function of the H/L ratio values are summarized in figures 2a and 2b, respectively. By using a model of spherical cap as a geometric approximation to the cell [5] the cellular volumes were estimated (Fig. 2c). The obtained values are in agreement with those achieved directly with the SensoSCAN S neox software, for which the cellular edges have been considered (differences lower than 5% in all cases).
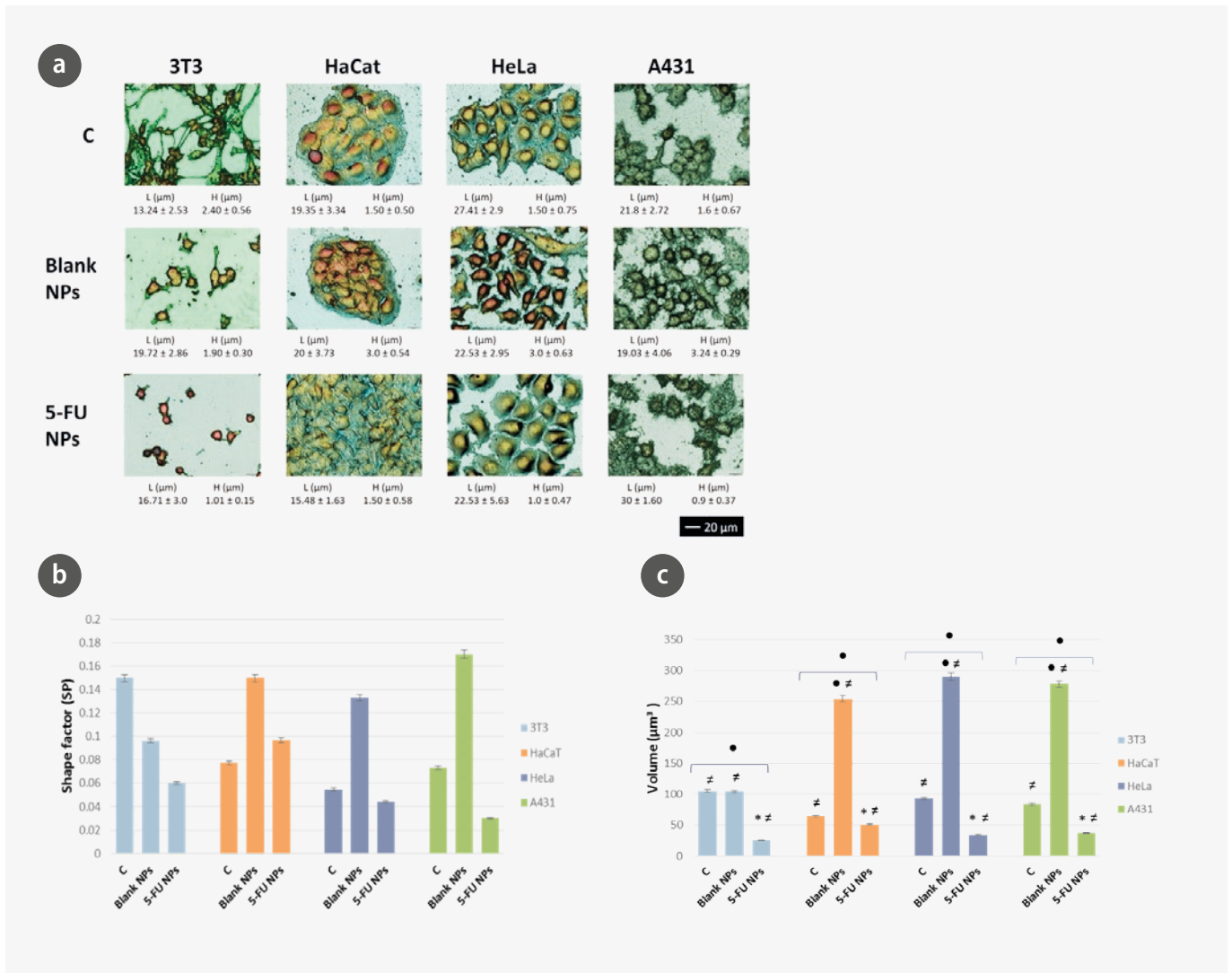
Fig. 2. Representative images of profilometry on Confocal mode (magnification 20x) and morphological parameters (L: length and H: height) (a) shape factor (H/L ratio) values (b) and estimation of cell volume from the data obtained from optical profilometry profiles and using a model of the spherical cap as a cellular geometrical approximation. Results are expressed as the average of ten independent cells ± standard deviation. *p<0.001 indicates significant differences against control cells, *p<0.001 indicates significant differences between treatments for the same cell line, and ≠p<0.001 indicates significant differences between cell lines for the same treatment. ("3D profilometry and cell viability studies for drug response screening" by M. C. Morán, F, Cirisano and M. Ferrari is licensed under CCC).

Conclusions

Using optical profilometry, analyses of cell morphology with response times compatible with conventional methods, like the MTT cell viability assay, were performed [6]. However, these analyses avoid some contradictory results due to changes in the metabolic activity that may appear by MTT action without alteration of the viable cell number. Besides, the measurement of cell viability results from the arithmetic means of the metabolic activity of 10^4-10^5 cells/mL. This large number of cells may hinder the determination of morphological changes in very early stages or small portions of the cell population.

The determination of single cell volume through 3D profilometry is an added value on establishing patterns or mechanisms of cell death, otherwise not detectable by a simple cell viability assay even on surfaces well over the limits of other techniques (cm^2). This technique can be regarded as an interesting tool for observing changes in cell morphology during the screening of potential drug and toxic materials, discriminating between the two greatest models of cell death especially in very short times in treatment procedure for an early prediction on success expectations of a therapeutical agent.

The success of research in biomedicine lies in the application of nanomaterials in the treatment of diseases as well as in the development of tools for their diagnosis. In this work, 3D optical profilometry, setting qualitative and quantitative analyses, meets the requirements for a screening tool of potential therapeutic or toxic drug delivery systems, stimulating cell growth or inducing cellular death. This work is the research team's first publication combining both disciplines (treatment and diagnosis).

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