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MICROFLUIDIC LIVE-IMAGING WITH CELLVIEWER TECHNOLOGY TO PERFORM BIOTECHNOLOGICAL TASKS

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***Abstract*— Cells grown in a monolayer tend to flatten in the lower part of the plate adhering to and spreading in the horizontal plane without expanding in the vertical dimension. The result is that cells grown in 2D have a forced apex-basal polarity. Microfluidic Live-Imaging with CellViewer technology is an ideal solution to observe the maintenance of a cell in excellent health, trying to bridge the gap between the 2D and 3D model. In this work we propose to test the system on a single isolated Jurkat cell in the microfluidic cartridge and record the timelapse for 4 hours. After adaptive autofocus, when sliding inside the cartridge chamber, the single cell is tracked under the action of the optics and the 3D rotation was experimentally successfully achieved. Then a single cell viability assessment was used using MitoGreen-dye a fluorescence marker selectively permeable to live cells. ImageJ software was used to: calculate the diameter of a single cell, create fluorescence intensity graphs along a straight line passing through the cell, visualize spatial fluorescence intensity distribution in 3D.**

***Keywords*—** Microfluidics; Biotechnology; Live-Imaging; Biomedical; Biomedical Enineering;

I. INTRODUCTION

The traditional 2D culture systems growth alone or in co-culture on plates, in which experiments supported by in vitro imaging are conducted for different functional, pharmacological, toxicological, and even clinical applications; they have long been widely used and already known for the nature of their cost and high repeatability. However, 2D culture systems cannot reach a stage of 3D organization equal to in vivo, due to the disadvantages associated with the lack of specific tissue architecture, mechanical-biochemical signals, cell-cells and extracellular matrix[1]–[3].

One issue with conventional 2D cell culture systems is the inadequate quality and quantity of Extracellular Matrix (ECM), which is fundamental to the support of the structure by facilitating communication between the different cell populations embedded in the matrix by imparting mechanical properties to the tissues[4]. Cells in 2D culture are not surrounded by ECM and therefore are different from the structure of an in vivo cell system, as they cannot: migrate, polarize, differentiate in response to[5]–[8]. Despite their proven value in biomedical research, 2D models cannot support differentiated and cell-specific functions in tissues or accurately predict in vivo tissue functions and drug and biological modulator activities [9]–[12]. These limitations have led to a growing interest in the development of more complex models, such as those that incorporate multiple cell types or involve cell modeling, and in three-dimensional (3D) models, which better represent the spatial and chemical complexity of living tissues[13]–[15]. The methods and timing of the research are expensive, the in vitro and ex vivo activity phase is of enormous importance to have information that can follow the next steps. Most of the research carried out has turned its attention to traditional and 3D methods, the analysis of these results is part of the economics of research. The optimization of available resources and the use of new approaches show new scenarios regarding the potential of laboratory research and the possibility of improving the quality of data and information.

A. 3D models

Some 3D models provide great results in representing tissue structures in the physiological field compared to two-dimensional 2D cell culture[6], [15]–[18]. The fabrics have a hierarchical structure that contains micro-architecture features

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that can be studied on many length scales. These include the subcellular/cellular scale (1–10 μm), which affects cellular function; the multicellular scale (10–100 μm), which determines the type and degree of intercellular interactions; and the tissue scale (100–1000 μm), which correspond[3], [19]. Deciphering population heterogeneities is a long-standing goal in cellular biology. At the level of single cells, such heterogeneities are usually observed at the genomic, transcriptomic or phenotypic levels[20]. In general, spheroids, self-organizing and heterogeneous cell aggregates up to 400–500 μm in size, are used for research, resulting from the suspension or adhesion on the single-cell jamb or co-culture of more than[21]. Spheroidal models have advantages derived from their geometry and the possibility of developing effects in co-culture and sustainability generally long-term, as they mimic optimal cell-cell-cell-ECM physiological interactions, reproducibility, the similarity in protein-gene expression profiles The use of these models is not transferable to cell types, as 3D spheroids of these cells tend to disintegrate or take unpredictable forms[21]. To avoid unpredictable and not currently useful developments, various types of scaffolds are manufactured and applied tools that control the development and structuring of spheroids' uniform dimensions[22].

The realization of an organoid, pseudo-organ, or neo-organ has in common some processes present in the various stages of development and formation of a living organism. This includes differentiation, proliferation, polarization, adhesion and precisely controlled apoptosis that combined with self-organization and multi-cellular pattern leads to the development of the various districts[22]. The Organoids or Tissues Organs are an in vitro 3D cell cluster derived from stem cells or progenitors and/or donors that spatially organize themselves in a similar way to their counterpart in vivo[23]. In the structuring and organizations of culture systems and especially of co-culture systems cells must maintain an adequate phenotype compatible with the external cellular environment and the duration of this phenomenon must be particularly protracted over time. For adhesion-dependent cells, interactions with the surrounding ECM and neighboring cells define the shape and organization of cells. One of the most surprising differences observed when comparing cell cultures in 2D and 3D is the morphological dissimilarity and their evolution over time. Cells grown in a monolayer tend to flatten on the bottom of the plate dish by adhering and spreading on the horizontal plane without expanding into the vertical dimension. The consequence is that cells grown in 2D have a forced apex-basal polarity. This polarity is probably relevant for certain cell types such as epithelial cells, but it is unnatural for most cells especially those of cubic or multifaceted type. The mesenchyme, if incorporated into a 3D ECM, take on a starry morphology and polarize only by bottom-up during migration[12], [24].

To support and guarantee the functional maintenance of a 3D structure, one must consider the structures and dynamics of regulatory networks, increasingly studied with live-imaging microscopy[25].

However, commercially available technologies that can be used for current laboratory needs are limited, although there is a need to facilitate the acquisition of cellular kinetics with a high spatial and temporal resolution, to elevate visual performance and consequently that of experimentation[6], [26]–[28].

B. **Microfluidic live-imaging**

2D models in Petri dishes allow for collective cell simulation and behaviors related to disease modeling and understanding but the advent of laboratory and organ devices on a chip shows that information obtained from 2D cell cultures on plates differs significantly from results obtained in microfluidic environments as they reflect more biomimetic aspects [30].

2D culture imaging does not allow to fully appreciate the morphology of the cell population and the three-dimensionality of the sample, one of the reasons could be the unappreciated evolutionary changes. The use of imaging is an essential requirement for the study of the structural and functional morphology of the neo-organ, of its positioning / polarization and of cell differentiation, allowing the in vitro modeling of even the most complex organs [31].A new technology based on newly developed microfluidics and imaging techniques can enable the management and identification of the phenotype, the biological activities of the present populations of the present populations without destroying the 3D of an organoid or derived in culture or co-culture of progenitor organs and/or donors who self-organize in space/time like the in vivo[23], [29], [30]. However complete lab-on-a-chip devices that can work with the automated procedure and allows to see the behavioral cities of cells or their alterations to support microfluidic system, have not been prevented in the literature usage[16].

Currently from what can be found in the bibliography, we have found that commercially the only equipment available to perform some specific protocols is CELLviewer[31].

The CELLviewer is a newly conceived and developed multi-technology instrumentation, combining and synchronizing the work of different scientific disciplines in the field of management of both simple and complex 3D culture systems, allows to maintain in the most natural conditions possible the three-dimensional structure, following it over time through high-definition time-lapse microscopy.

II. CELLVIEWER

A. ***What is CELLviewer?***

The CELLviewer, Fig. 1., is a lab-on-a-chip for cells or neo-organs to be managed in the absence of adhesion, designed by CellDynamics, Bologna Italy. This multi-technological system is composed of a hardware tool and disposable parts: microfluidic chips used to insert the sample into them. The device's specific capabilities include environmental control, automatic change of cultural media, the ability to insert individual cells or neo-organs, and perform an optical analysis in the light field, darkfield, and fluorescence microscopy.