A viability study of 3D tumor spheroids after their mass-density characterization *via* an innovative flow-based biophysical method

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Abstract

The simple measurement of mass density, size and weight of sphere-like 3D cell models has been recently enabled by a specifically conceived flow-based method. Here we demonstrate that such technique also allows the post-analysis collection of live 3D tumor spheroids, without compromising their viability.

Introduction

It is highly acknowledged that 3D cell cultures present countless advantages when compared to 2D approaches. However, sample heterogeneity, as well as the refinements of standardization methods, still represent crucial areas of improvement for 3D cell models. Moreover, chemical, mechanical and physical developments are needed to continuously adapt state-of-the-art technologies to the evolution of 3D cell models over time.[1-4] For such samples, knowing physical parameters like mass density, is revealing its importance in growing *in vitro* tumor cells clusters, which represents a crucial area of interest in 3D biology.[5] This would help in studies ranging from anticancer drugs to the elucidation of tumor onsets and progressions.[6,7] Here we analyzed 3D tumor spheroids *via* a flow-based technique that allows measuring mass density, size and weight of sphere-like 3D cell aggregates, based on gravimetric and sample-tracking analysis combined.[8] This allowed us to present a comparative viability study over time, performed through a metabolic assay, of physically characterized and collected samples *vs* controls.

Materials and Methods

Spheroids of the human breast cancer cell line MCF7 were generated into U-bottom ULA 96-well plates (Greiner Bio-One GmbH). MCF7 were seeded at the concentration of 700 cells/well in two 96-well plates, and cultured for 7 days in DMEM (Corning® Life Sciences) with 10% FBS (Gibco™, Thermo Fisher Scientific) at 37 °C and 5% CO_2. On day 7 of formation, 144 fully mature and organized spheroids were collected into a 15 mL centrifuge tube, then washed and resuspended in 7.5 mL of W8 Analysis Solution (WAS, CellDynamics). Samples were analyzed using the W8 Physical Cytometer, (CellDynamics) according to the previously presented flow-based method.[8] Briefly, the method allows calculating the terminal velocity of a free-falling sample, positioned into a vertical flow-channel, when the WAS is at rest. This is achieved through a Stokes' law adaptation, combined with a shape recognition algorithm and a sample-motion tracking (Figure 1). The circular reference, assigned to each image frame of the falling sample, allows the extrapolation of the average radius used for the physical calculations.[8]

For the viability test, three independent experiments were performed and the mean values of the physical outputs were extrapolated from two repetitions. Twenty spheroids were analyzed and collected for each experiment. The Shapiro–Wilk statistical test was performed to analyze the distribution of the dataset. The collected spheroids (SAMPLE) were compared to spheroids maintained at room temperature in WAS as negative control (CTRL–), along with spheroids kept in culture medium at 37°C and 5% CO_2 as positive control (CTRL+). The AlamarBlue™ assays were performed for all conditions according to manufacturer's instructions, and fluorescence intensity (FI) at 590 nm was monitored up to 72h. FI delta values represent ratios between consecutive timepoints (24, 48, 72h).

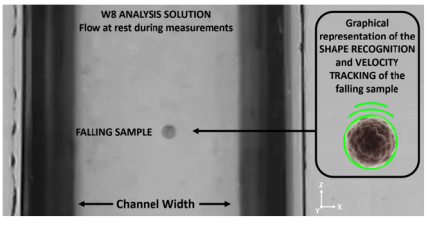

Figure 1. Bright field image of a representative falling sample and graphical representation of its shape recognition and velocity tracking performed by the W8 Physical Cytometer.

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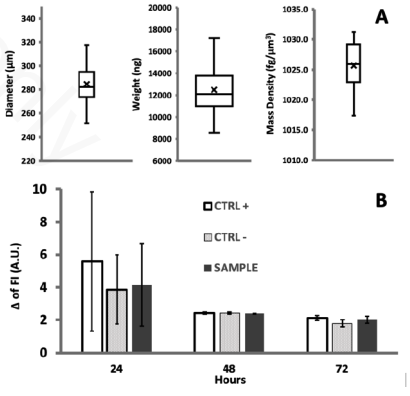

Figure 2. A) Diameter, weight and mass density values of MCF7 spheroids. B) AlamarBlue™ fluorescence intensity (FI) of CTRL+, CTRL–, and SAMPLE (white, grey and dark grey bars respectively) expressed as delta values between consecutive timepoints (24, 48 and 72h). Data are presented as the mean value ± SD of three independent experiments. Statistical analysis was performed using two-tailed unpaired Student's t-test (P>0.05).

Results

As displayed in Figure 2A, live MCF7 spheroids showed an average diameter, weight and mass density of 285 ± 16 µm (left), 12491 ± 2100 ng (center) and 1025.7 ± 4.1 fg/µm³ (right), respectively. The viability of samples collected after the physical characterization (SAMPLE) was investigated and compared with CTRL+ and CTRL–. Figure 2B clearly shows that the flow-based analysis does not alter spheroids' viability. Specifically, no statistically significant differences were found between SAMPLE and controls (CTRL+ and CTRL–), at any of the 3 analyzed timepoints (24, 48 and 72 hours). Of note, the doubling of FI delta values over time demonstrates the preserved metabolic activity.

Discussion and Conclusions

The adopted flow-based method constitutes a label-free and non-invasive solution to perform the physical characterization of live spheroids, without compromising their viability. Therefore, the collected samples are prone to be re-plated or exploited for further downstream analysis. This represent a great advantage when compared to techniques such as super-resolution microscopy, flow cytometry or immunohistochemistry, which are meant to be end-point assays. Furthermore, the recovery of specifically selected subsets of viable spheroids may significantly improve standardization- and compactness-related studies of 3D cell models. This would potentially pave an alternative way to increase our knowledge of complex 3D samples like organoids.

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