

Effective Determination of Cellular Viability in Microtissue Spheroids

ABSTRACT

Important cell-to-cell interactions can only be recreated in vitro by 3-dimensional cell culture models, preferably in scaffold free systems. Advanced 3D in vitro cell culture systems such as those provided by InSphero are gaining attraction in biopharmaceutical research as they become available in standard microtiter plate formats. This article demonstrates that the CellTiter-Glo® Luminescent Cell Viability Assay with its "add, mix, measure" format, is ideally suited for the assessment of viability in spheroid cultures.

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Introduction

Biology is commonly defined by a complex sequence of a multitude of biochemical reactions within a cell but also by the interplay of cells with their direct neighboring cells. In higher order organisms, cell-to-cell interactions are crucial to fulfil concerted, tissue- or organ-specific tasks. Such important cell-to-cell interactions can only be recreated in vitro by 3-dimensional cell culture models, preferably in scaffold free systems. The vast majority of primary cells and cancer cells spontaneously form spheroids when cultivated in suitable formats, such as the hanging drop or microgravity rotating vessel system.

Spheroid cultures are gaining attraction in biopharmaceutical research as they become available in standard microtiter plate formats and thus can be handled by lab robotic systems. Even though spheroid cultures are of higher biological complexity than 2-dimensional cell culture, most often cell-based biochemical assays can be performed in an identical manner as with regular cell cultures.

Cell viability is a crucial end point in cell-based in vitro screening for the measurement of toxicity or cell proliferation. The CellTiter-Glo® Luminescent Cell Viability Assay (Cat.# G7570) represents an ideal tool for the assessment of viability in spheroid cultures as it provides a convenient "add, mix, measure" format. The assay uses the luciferase reaction to measure ATP, a global indicator of cellular metabolism. The substrate mix contains luciferase, which in the presence of Mg2+ and ATP converts luciferin into oxiluciferin and concomitantly releases energy in the form of luminescence. Signal strength is directly proportional to the amount of ATP present, which correlates with the metabolic activity.

Linearity of ATP Signal and Spheroid Size

The human glioblastoma cell line SNB-19 was used to create microtissues of different sizes, starting from 250 up to 10,000 cells per microtissue at the time point of seeding. Spheroid formation was conducted in the InSphero GravityPlus™ hanging drop culture platform. Four days after inoculation, the microtissues were transferred into standard V-bottom plates and assessed for average spheroid size.



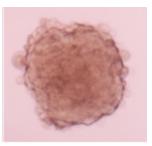


Figure 1. SNB-19 human glioblastoma cells. Cells were grown in standard 2D culture (left panel) or after spheroid formation in the hanging drop culture platform GravityPlus™ (InSphero; right panel).

The curvilinear relationship between the spheroid size and the seeding cell number is related to different proliferation rates during the microtissue formation process (Figure 2). This phenomenon is caused by contact inhibition of cancer cells, which is a function of spheroid size or the number of cells initially inoculated in a hanging drop.

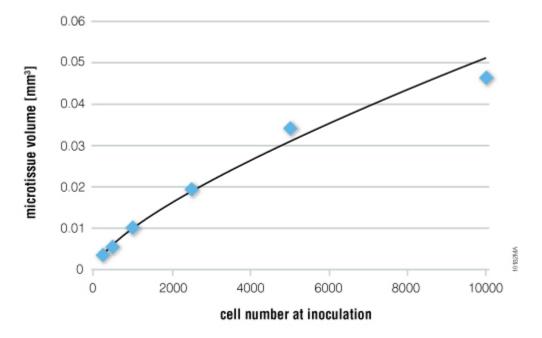


Figure 2. Microtissue volume and inoculation cell number relationship of SNB-19 spheroids 4 days after seeding into hanging drops. The microtissues were considered as idealized spheres and volumes were calculated according to the sphere formula $V=(4/3)\pi R3$.

The microtissues were subjected to ATP measurement with the CellTiter-Glo® Luminescent Cell Viability Assay according to the protocol in Technical Bulletin #TB288. As a slight adaptation to the specifics of spheroid culture, lysis with the CellTiter-Glo® Reagent was prolonged to 20 minutes and enhanced by pipetting up and down at the moment of substrate addition and

prior to the transfer into an opaque white tissue culture plate. Luminescence was measured with a TECAN™ Infinite 200 Promicroplate reader. The sensitivity was set to 1000ms.

The assay shows a good correlation between the luminescent signal and the number of cells that were initially seeded into hanging drops (Figure 3). The deviation from a completely linear relationship either indicates a decreasing metabolic activity or a reduced proliferation rate as a function of the spheroid size.

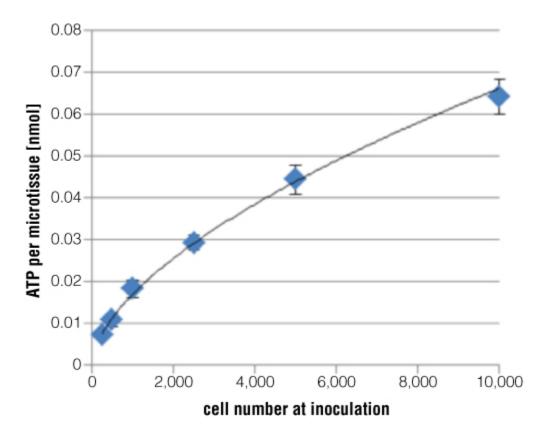


Figure 3. Relationship of ATP content and initial cell seeding number for SNB-19 human glioblastoma cells. Microtissues started with initial cell seeding number of 250, 500, 1000, 2500, 5000, and 10,000 cells per drop were measured for luminescence and signals were calculated for ATP content by referring to an ATP standard measurement. Data represent the mean ±S.D. of 5 replicates for each microtissue size. The signal follows a curvilinear relationship between the ATP content and the number of initially seeded cells per microtissue (r2=0.99).

When the intracellular ATP content is plotted against the measured microtissue volumes, an almost perfect linear correlation is observed.

As the volume of a microtissue is directly correlated to the number of cells, the linear relationship in Figure 4 demonstrates that ATP is a reliable indicator for the amount of cells in a given microtissue.

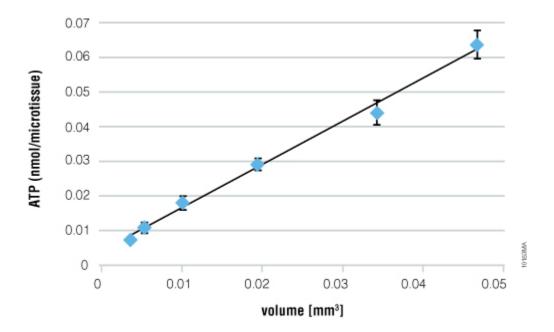


Figure 4. Relationship between SNB-19 sphereoid size and ATP content. A perfect linear relationship is shown between the size of SNB-19 spheroids seeded at 250, 500, 1000, 2500, 5000, and 10,000 cells/drop) after a 4 day culture and their intracellular ATP content as assessed with the CellTiter-Glo® Luminescent Cell Viability Assay (r2=0.99).

To demonstrate that the ATP signal is directly correlated to the microtissue cell number, the microtissue DNA content was assessed by Picogreen® staining subsequent to the ATP measurement. Figure 5 displays a perfect linear relationship of the microtissue ATP signal and the DNA content. This convincingly shows that ATP is not only an indicator for metabolic activity but also serves as a measure for the number of cells in spheroid culture, provided cell viability is not compromised.

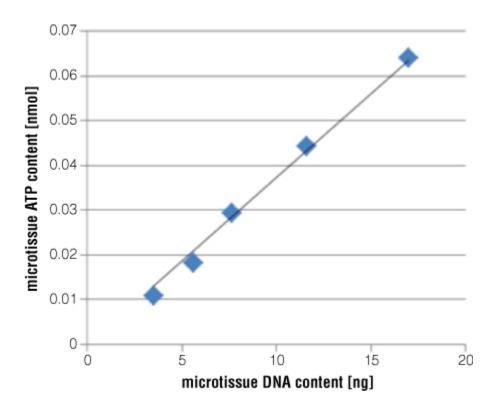


Figure 5. ATP content correlates with DNA content. SNB-19 microtissues were first analysed for ATP content with the CellTiter-Glo® Luminescent Cell Viability Assay. Thereafter the lysate was incubated with the nucleic acid stain Picogreen for quantitating double stranded DNA. The strong linear relationship (r2=0.99) between ATP and DNA content underscores the suitability of ATP as a measure of microtissue cell count.

Assessing Drug-Dependent Cytotoxicity

To demonstrate that the measurement of ATP is a suitable marker for drug induced cytotoxicity in tumor spheroids, HCT-116 colon carcinoma cells were seeded for the generation of microtissues and thereafter incubated in the presence of increasing concentrations of the cell toxin Staurosporin.

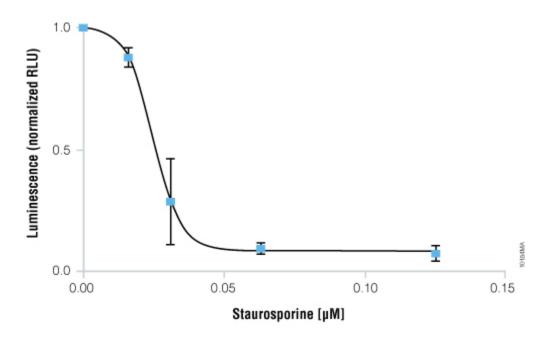


Figure 6. Dose response curve of HCT-116 microtissues treated with Staurosporin. HCT-116 cells were seeded in the hanging drop culture platform GravityPlus™ (InSphero) at a density of 500 cells/drop. After 3 days the microtissues were treated with Staurosporin for 24 hours and assayed for intracellular ATP with the CellTiter-Glo® Luminescent Cell Viability Assay.

This result clearly demonstrates that the ATP viability assay can be applied to spheroid culture for the measurement of cell toxicity just as easy and straight forward as with conventional 2D cell culture. This is also facilitated by the fact that InSpheros' microtissue cultures are completely free of any kind of scaffold that would eventually interfere with cell-based biochemical read outs.

Conclusions

The strong correlation between DNA and ATP supports the notion that cellular ATP content, aside from being an indicator of cell viability, serves as a good surrogate measure for the determination of cell number. Due to its high sensitivity, the CellTiter-Glo® Luminescent Cell Viability Assay proves to be ideally suited for the concept of one microtissue per well with a total cell number as low as a few hundred per data point.

As opposed to other reduction of tetrazolium salt-based cell viability assays, the CellTiter-Glo® ATP assay can be performed on tissue lysate with comparatively short incubation times due to instant access to the ATP. The CellTiter-Glo® Assay perfectly suits the needs for routine viability/cytotoxicity testing with advanced 3D in vitro cell culture systems, in particular with scaffold-free microtissues as provided by InSphero. Highly relevant biological cell culture models used in conjunction with easy to use, sensitive and reliable cell-based assay systems will be tremendously helpful in biopharmaceutical research to obtain predictive results in a fast and less expensive manner.

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