

High throughput quantification of HepG2 spheroid formation on ultra-low attachment plates

introduction

Research to identify new anticancer drugs is currently facing significant challenges, as only 5% of compounds that show efficacy in pre-clinical development go on to become licensed drugs. Traditionally 2D cell culture models have been employed to evaluate drug candidates in the early phases of the drug discovery process, however, there is increasing evidence that cells grown in 2D monolayers do not accurately reflect the biological complexity of tumours. The requirement for better *in vitro* tumour models that are compatible with high throughput screening campaigns has led to the development of 3D cell culture models, especially multicellular tumour spheroids, which retain many of the morphological and genetic traits of tumours.

This application note describes how highly uniform HepG2 spheroids can be grown in the wells of ultra-low attachment plates. We then show how the acumen Cellista can be used to rapidly and accurately quantify spheroid size in response to doxorubicin drug treatment.

The acumen Cellista laser scanning imaging cytometer offers rapid (5 minutes per plate) whole well image acquisition in a wide range of plate types (24- to 1536-well plates). The acumen Cellista enables a wide range of fluorescent reagents to be combined in multicolour, multiplexed assays, as it is equipped with a choice of 405, 488, 561 and 640 nm lasers and able to simultaneously acquire up to four channels of fluorescence data per laser.

methods

cell culture

HepG2 cells are cultured to approximately 80% confluency, washed with PBS and harvested using trypsin-EDTA. The cells are then centrifuged at 160g for 6 min and the cell pellet is re-suspended in an appropriate volume of complete growth medium for seeding into the wells of ultra-low attachment plates. For 96-well plates (Corning #4520) 300 cells in 200 μ L of medium are seeded per well; for 384-well plates

(Corning #3830) 300 cells in 50 μ L of medium are seeded per well. The cells are cultured at 37°C / 5% CO₂ in a humidified incubator for a total of 6 days. 24 h after seeding, the cell suspension will have assembled into a single 3D spheroid located centrally in the well. 72 h after seeding, 50% of the medium is removed from the wells and replaced with fresh media containing doxorubicin (2x concentrated, final concentrations 316 pM - 3.16 μ M). Prior to imaging, the spheroids are stained with 2 μ M Calcein-AM for 30 minutes at 37°C.

image acquisition

Whole well TIFF images were acquired using the acumen's 488 nm laser excitation with the FL-2 detection channel (500-530 nm) for Calcein-AM. The acumen's optics can rapidly image the entire well area, immediately reporting out the spheroid area and spherical volume.

key benefits

acumen[®] Cellista provides the fastest way to perform high content tumour spheroid analyses. This simple and robust method:

- grows individual 3D spheroids in the wells of ultra-low attachment plates
- rapidly (5 minutes/plate) determines spheroid size and volume for whole wells
- simultaneously gives a measure of cell viability following drug treatment



results and analysis

a 96-well plate

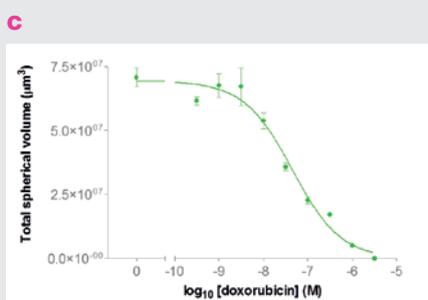
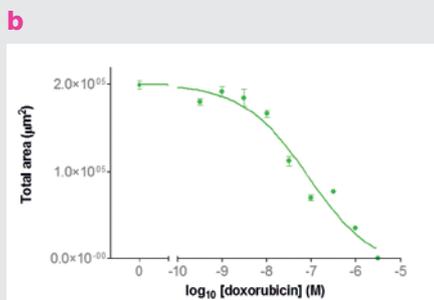
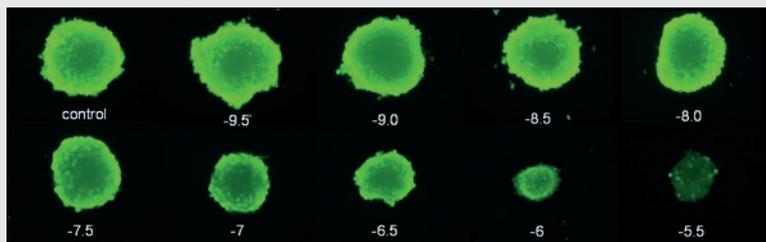


Fig 1.

a Whole well TIFF images of HepG2 tumour spheroids grown on 96-well ULA plates. The amount of doxorubicin added to each well shown on each image as \log_{10} [doxorubicin] (M).

b - c Drug concentration-response curves (mean \pm SEM, n=6)

a 384-well plate

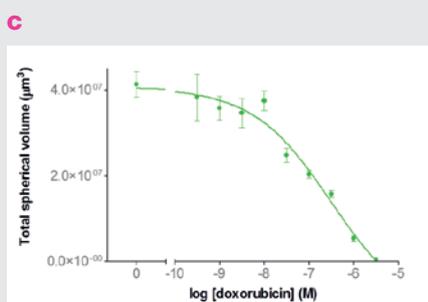
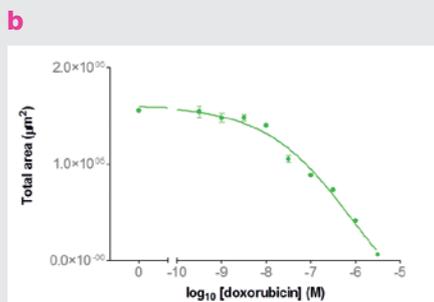
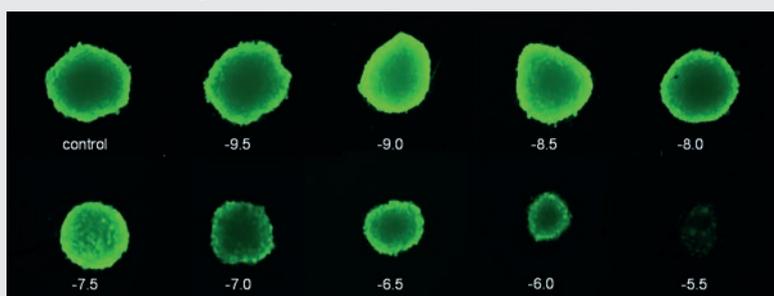


Fig 2.

a Whole well TIFF images of HepG2 tumour spheroids grown on 384-well ULA plates. The amount of doxorubicin added to each well shown on each image as \log_{10} [doxorubicin] (M).

b - c Drug concentration-response curves (mean \pm SEM, n=8)

conclusion

We have established a simple and robust method to grow tumour spheroids in 96-well and 384-well microplate formats. Calcein-AM staining of tumour spheroids followed by imaging on the acumen Cellista allows the determination of spheroid size by area and spherical volume, and also provides a measure of cell viability following drug treatment. This method provides a robust and reproducible assay readout, as proven by consistent drug concentration-response curves. These data show that the acumen Cellista is ideally suited for the rapid (5 minutes/plate) high throughput analysis of tumour spheroids.

about acumen

TTP Labtech's acumen is a laser scanning imaging cytometer designed to provide immediate whole well, content-rich cytometric and image-based analysis. Its F-theta lens gives a uniform illumination across the field of view with a large focussed depth of field, which enables high throughput, whole well image acquisition across a range of plate types. acumen enables a wide range of fluorescent reagents to be combined in multicolour, multiplexed assays. Its easy-to-use, template-driven software offers an industry-proven route for quick adoption across a wide range of applications.

key capabilities

- whole well imaging means all spheroids are counted
- spheroid volumes are determined without z-stacking
- provides a measure of cell viability following drug treatment
- robust data is obtained in a little as 5 minutes per plate

get in touch

TTP Labtech Ltd

Melbourn Science Park, Melbourn
Hertfordshire SG8 6EE, United Kingdom
Tel: +44 (0) 1763 262 626
Fax: +44 (0) 1763 261 964

TTP Labtech Inc

One Kendall Square, Suite B2303
Cambridge MA 02139-1594, United States
Tel: +1 (617) 494 9794
Fax: +1 (617) 494 9795