Rapid analysis of 3D tumour spheroids in soft agar and on ultra-low attachment plates using a laser scanning imaging system

Anne F Hammerstein, Diana Caracino, and Paul Wylie

TTP Labtech Ltd, Melbourn Science Park, Melbourn, Hertfordshire, Royston, SG8 6EE, UK

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[1]. 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* models that are compatible with high-throughput screening campaigns has led to the development of 3D cell cultures models, especially muliticellular spheroids, which retain many of the morphological and genetic traits of tumours.

Here we describe the formation of such spheroids by two methods: on ultra-low attachment plates and in semi-solid agarose. Both methods are compatible with 96- and 384-well microplate formats.

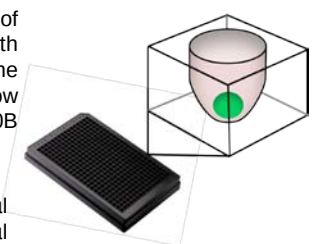
We then used the acumen to rapidly image entire microplates (5 minutes/plate), reporting a range of parameters such as spheroid number, area and volume. The acumen is ideally suited to the high content analysis of spheroids, as the whole-well scanning capability of the instrument will include data from all the spheroids in a well, while the large depth of field of the scan lens allows the determination of individual spheroid volume without the need to acquire a Z-stack of images.

[1] Hutchinson et al., Nature Rev. Clin Oncol.

methods

A tumour spheroid formation on ultra-low attachment plates.

- Single cell suspensions of HepG2 cells in complete growth medium were seeded into the wells of Corning ultra-low attachment plates (#3090B #3091C)
- Seeding densities:
  96-well plate: 300 cells in 200 µl
  384-well plate: 300 cells in 50 µl

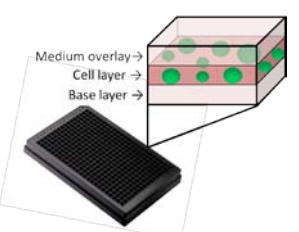


- Within 24 hours the cell suspension assembled into a single 3D spheroid located centrally in the well
- After 72 hours, 50% of the media was removed from the wells and replaced with fresh media containing doxorubicin (2x concentrated, final concentrations 316 pM - 3.16 µM)
- The spheroids were cultured for a total of 6 days

B tumour spheroid formation in soft agar

	96-well	384-well
Base layer (0.6% agarose in medium)	25 µl	10 µl
Cell layer (single cell suspension in 0.4% agarose/medium)	500 cells in 50 µl	150 cells in 20 µl
Medium overlay	75 µl	30 µl

- Spheroids were cultured at 37°C
- After 24 hours, doxorubicin stocks were added to the wells (5 µl, final concentrations 1nM - 1 µM)
- The spheroids were cultured for a further 4 days, with an overlaying media/doxorubicin change 3 days after seeding



C staining & imaging

- The tumour spheroids generated by either method were stained by adding calcein-AM (5 µM final) to the wells, followed by incubation for 1 hour at 37°C
- The plates were then imaged on the acumen (5 minutes for imaging and data export per plate)
- Data analysis was performed “on-the-fly”, reporting out the number of spheroids, area and volume

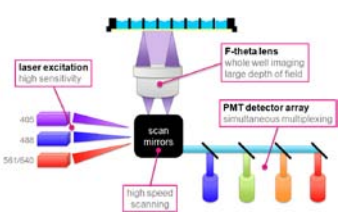


Figure 1. The acumen can sequentially scan with up to 3 lasers providing similar wavelength excitation to that of white light sources. PMTs detect up to 4 colours simultaneously.

results: spheroids grown on ultra-low attachment plates

A 96-well plate

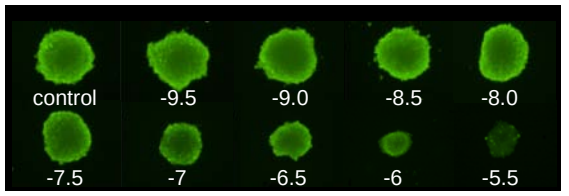
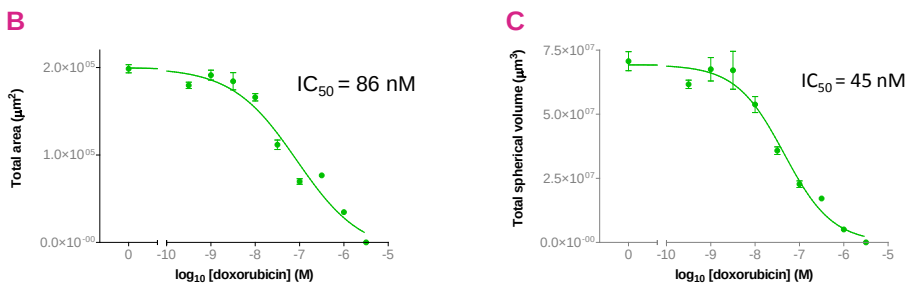


Figure 2A. Whole well TIFF images of HepG2 tumour spheroids grown on 96-well ULA plates. The amount of doxorubicin added to each well shown on each image as log_{10} [doxorubicin] (M) .
B-C. Drug concentration-response curves (mean ± SEM, n=6)



A 384-well plate

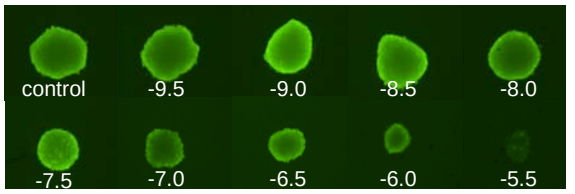
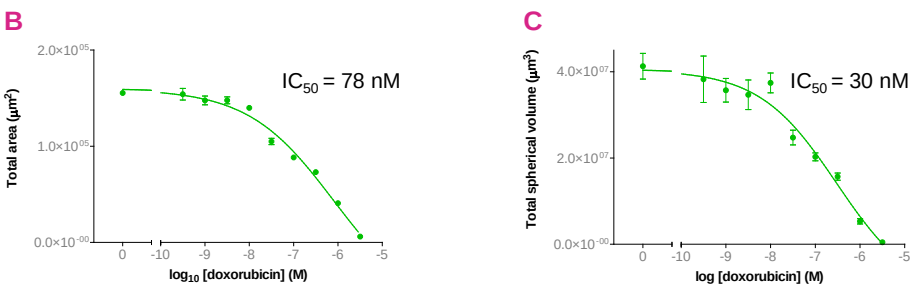


Figure 3A. Whole well TIFF images of HepG2 tumour spheroids grown on 384-well ULA plates. The amount of doxorubicin added to each well shown on each image as log_{10}[doxorubicin] (M) .
B-C. Drug concentration-response curves (mean ± SEM, n=8)



results: spheroids grown in soft agar

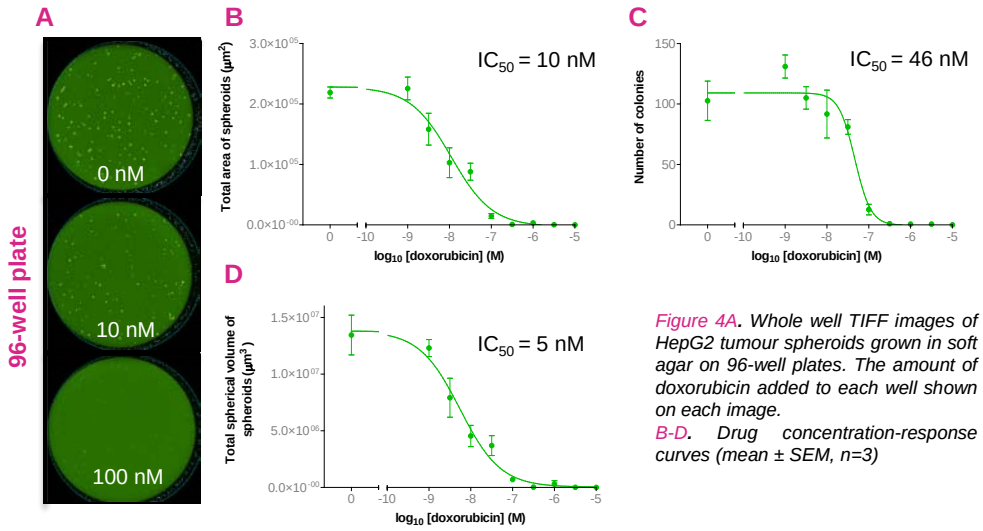


Figure 4A. Whole well TIFF images of HepG2 tumour spheroids grown in soft agar on 96-well plates. The amount of doxorubicin added to each well shown on each image.
B-D. Drug concentration-response curves (mean ± SEM, n=3)

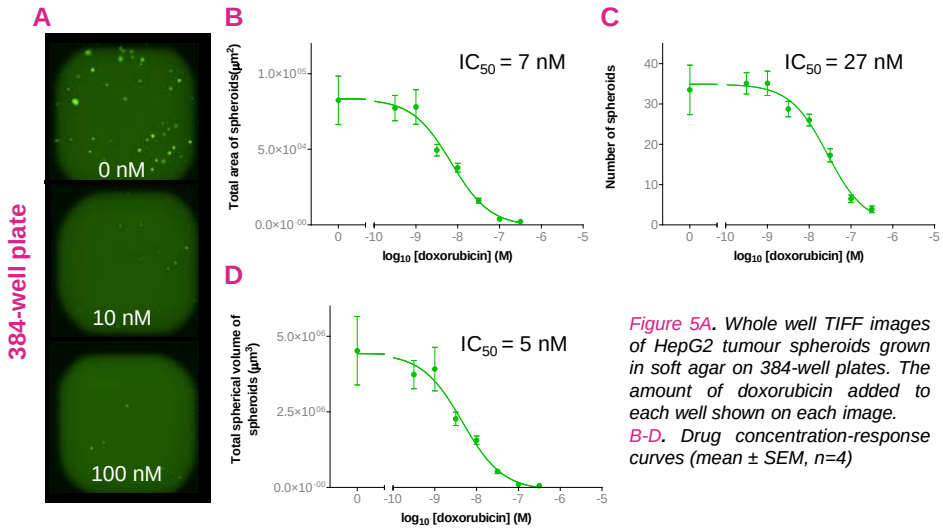


Figure 5A. Whole well TIFF images of HepG2 tumour spheroids grown in soft agar on 384-well plates. The amount of doxorubicin added to each well shown on each image.
B-D. Drug concentration-response curves (mean ± SEM, n=4)

TTP Labtech Ltd
Melbourn Science Park
Melbourn
Hertfordshire SG8 6EE
United Kingdom
tel: +44 1763 262626
fax: +44 1763 261964
sales@ttplabtech.com

TTP Labtech Inc
One Kendall Square
Suite B2303
Cambridge MA 02139
United States
tel: +1(617) 494 9794
fax: +1(617) 494 9795

conclusions

- we have established two simple and robust methods to grow tumour spheroids in 96-well and 384-well microplate formats
- calcein-AM staining of tumour spheroids followed by imaging on the acumen allows the determination of a range of parameters, such as spheroid number, area and volume, and also provides a measure of cell viability following drug treatment
- each method provides a robust and reproducible assay readout, as proven by consistent drug concentration-response curves
- these data show that the acumen is ideally suited for the rapid (5 minutes/plate) high-throughput analysis of tumour spheroids