Application Note

Improved toxicology prediction by utilising hepatic spheroids

- Improved outcomes for drug toxicity analysis using 3D culture generated in a ClinoStar compared to flat culture.
- Continuous testing and prolonged exposure to toxicological compounds can be conducted.
- The ClinoStar system can generate several hundreds of uniform spheroids
- High biomass can be produced for downstream analysis

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Introduction

Primary human hepatocytes are the gold standard of drug toxicology testing. However, this notion is challenged by high prices, donor variation and decreased tissue function in flat culture [1]. 3-dimensional cultures enable key liver functions to be regained, unlike 2D culture [2]. Generating spheroids from a hepatic cell line using the ClinoStar system allows creation of uniform constructs which can more reliably mimic in-vivo standards of toxicological determination [3].

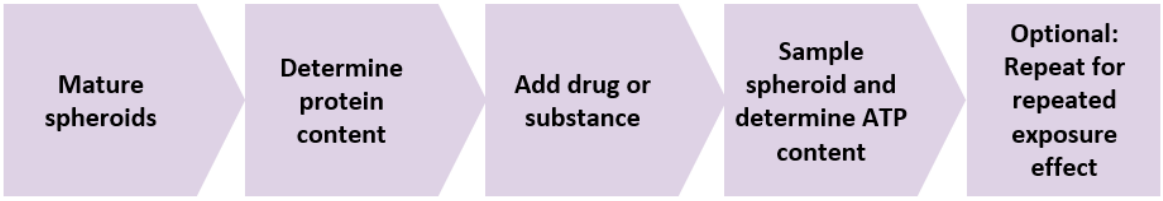


Figure 1. Schematic of the workflow determining toxicity

Reagents and Materials

- Low adherent microwell plate
- HepG2/C3A cells (ATCC HB-8065)
- ClinoReactor®
- ClinoStar®

Protocol

Maintenance

1. Change media thru the top port using needle and syringe three times a week.
2. Increase rotation speed as spheroids grow to ensure optimal media flow.

Expose spheroids to drug of interest normalised to amount of protein.

1. Remove ClinoReactor from ClinoStar and place in microscope.
2. Capture images of spheroids directly in ClinoReactor without sacrificing spheroids.
3. Determine surface area (planimetry).
4. Translate surface area to protein content using a lookup table found in supplementary data of reference [3].
5. Calculate concentration in media necessary for correct dosage ratio to protein amount per ClinoReactor®.
6. Expose spheroids to drug by media change.

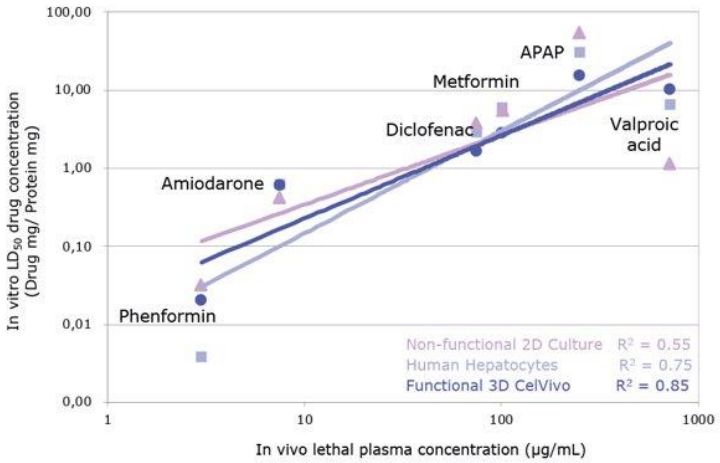
Determine drug effect on viability (ATP determination)

1. Perform ATP assay following manufacturer's instructions. We have adapted our protocol to CellTiter-Glo® from Promega.
2. Prepare standard curve dilutions in Hanks Balanced Saline Solution (HBSS).

3. Sample spheroids from ClinoReactor® into plate for assay. Our protocol uses a 96 well plate.
4. Remove growth media from well containing spheroids and add 100 µL HBSS.
5. Add standards to plate.
6. Add 100 µL assay reagent to each well, also standards.
7. Using a multi-pipette, destroy spheroids to release ATP.
8. Investigate if spheroids are destroyed using microscope. If not repeat step 7.
9. Remove bobbles and shake plate for 40 minutes protected from light.
10. Determine luminescence intensities using plate reader.
11. Calculate ATP concentrations of spheroids using standard curve.
12. Follow manufacturers manual for protein determination using e.g BCA assay.

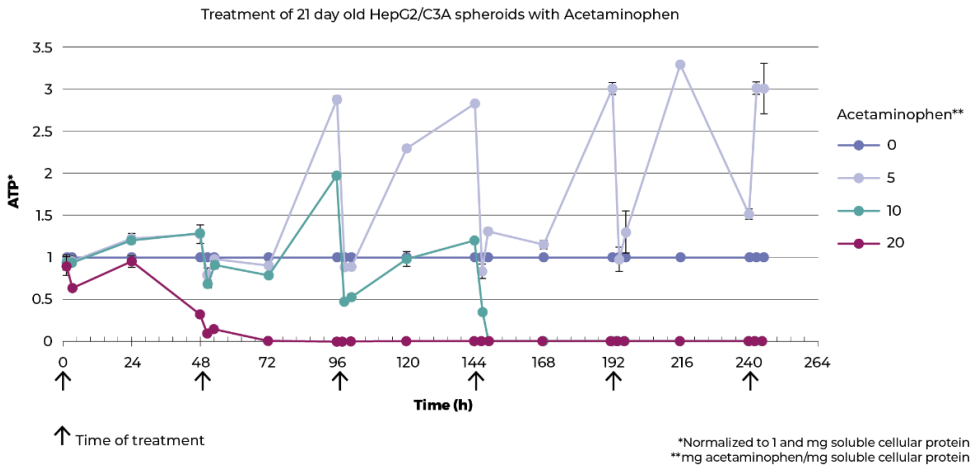
Determine drug effect on viability and normalise to protein content.

Figures and Data



Comparison of the predictability of flat culture (2D), primary human hepatocytes and 3D cultured C3A cells (as described in protocol). Here is used six common drugs and the relation between lethal plasma concentration. Based on R^2 values HepG2/C3A cells cultured in ClinoReactor® has the best predictability of these six drugs. Even better than primary human hepatocytes. [3]

ClinoReactor culture can also be used for repeated dosage of drugs to determine the long-term effects on as showed here viability (ATP content). Here 21 days old spheroids are dosed for 11 days. [3]



References

[1] P. Godoy et al., "Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME," Archives of toxicology, vol. 87, no. 8, pp. 1315–1530, Aug. 2013, doi: 10.1007/S00204-013-1078-5.
[2] K. Białkowska, P. Komorowski, M. Bryszewska, and K. Miłowska, "Spheroids as a Type of Three-Dimensional Cell Cultures-Examples of Methods of Preparation and the Most Important Application," International journal of molecular sciences, vol. 21, no. 17, pp. 1–17, Sep. 2020, doi: 10.3390/IJMS21176225.
[3] S. J. Fey, B. Korzeniowska, and K. Wrzesinski, "Response to and recovery from treatment in human liver-mimetic clinostat spheroids: A model for assessing repeated-dose drug toxicity," Toxicology Research, vol. 9, no. 4, pp. 379–389, 2020, doi: 10.1093/TOXRES/TFAA033.