Application Note

How to culture uniform mini liver-like constructs from HepG2/c3a using the ClinoStar system

Purpose

- This application note details a protocol using the ClinoStar and ClinoReactor system to generate spheroids that are uniform in appearance and function, with consistently reproducible results.
- ClinoReactor and ClinoStar culture systems enable a dynamic culture that can be maintained in a closed, contamination-free environment for longer periods of time.
- Our culture methods produce 3D spheroids that are capable of accurately mimicking *in vivo* function compared to 2D cultures.

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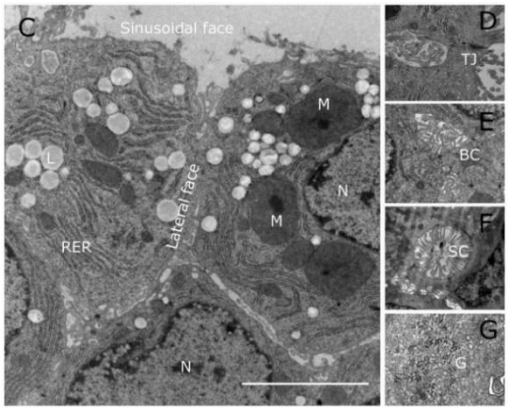
Introduction

3-dimensional cell culture has gained profound interest over the last few years [1]. Culturing cells as spheroids is significantly advantageous. Spheroids consist of complex cellular networks, allowing for decreased rates of proliferation. This enables the culture to spend its energy establishing cell-to-cell contact and communication, which permits increased *in vivo* like performance and function compared to standard flat culture [2].



Electron microscopy of 21 days old spheroids exhibiting structures seen *in vivo* **(C)**. Tight junctions **(TJ in D)**. Bile canaliculi-like structures **(BC in E)**. Sinusoid-like channels packed with long micro-villi **(SC in F)**. Glycogen granules **(G in G)**.
N, nucleus; M, mitochondria; RER, rough endoplasmic reticulum.
Scalebar indicates 5 µM.

Reagents and Materials

- Low adherent microwell plate
- HepG2/C3A cells (ATCC HB-8065)
- Growth media
- Hanks balanced saline solution
- Trypsin 0.05%
- Fetal bovine serum (FBS)
- Falcon tube
- Syringe
- Needle
- ClinoReactor®
- ClinoStar®

Protocol

Preparation of plate

1. Prepare microwell plate according to manufacturer's manual.

Sub-cultivation of HepG2/C3A cells

1. Aspirate growth media from cell culture flask.
2. Wash HepG2/C3A cells in flasks 2x using Hanks balanced saline solution.
3. Add appropriate amount of trypsin and incubate for 5 minutes at 37°C.
4. Add FBS to stop trypsinisation.

5. Transfer this solution to a falcon tube.
6. Wash cell flask with appropriate amount of growth media and transfer to falcon tube.
7. Centrifuge falcon tube for 5 minutes at 130xG.
8. Remove supernatant and resuspend in appropriate amount of growth media.
9. Perform cell count.

Generation of initial spheroids in microwell plate

1. Remove microwell plate from incubator for seeding.
2. Seed each well with 1000 cells/microwell. Add media up to a final volume of 2 mL .
3. Centrifuge plate for 100xG for 3 minutes and check uniform distribution of cells by microscopy.
4. Incubate plate for 24 hours (37°C, 5% CO_2, humidified).

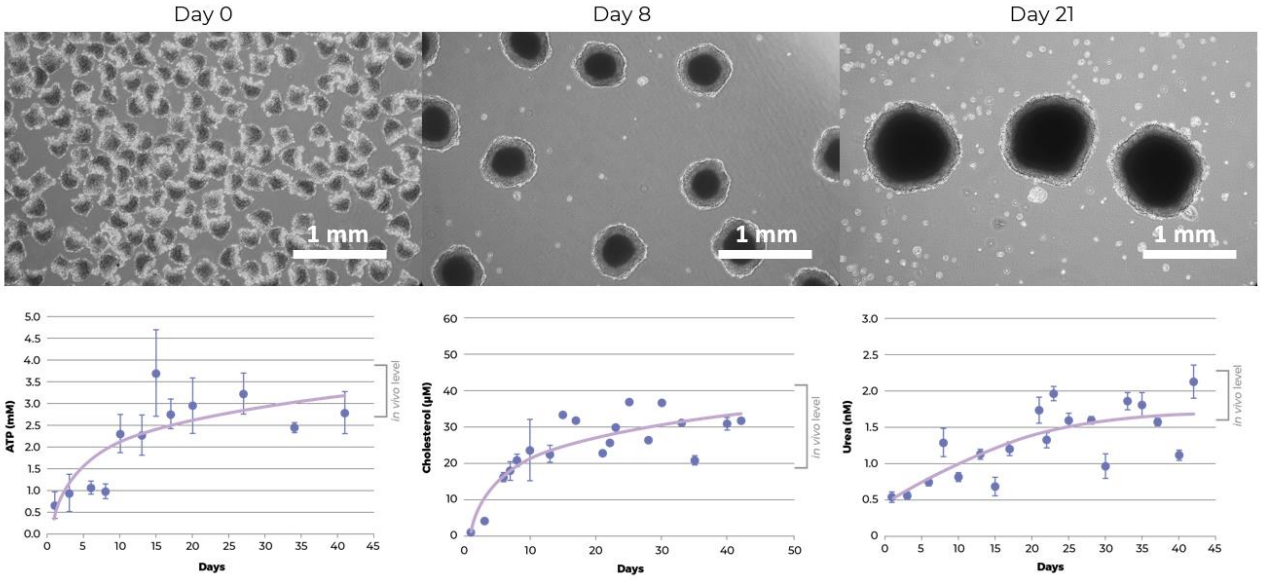
Transfer of spheroids from plate to ClinoReactor®

1. Humidify and equilibrate ClinoReactor® before use (consult 003_Protocol_Preparation_of_Clinoreactor).
2. Remove equilibration media from ClinoReactor and add 5 mL culture media of choice.
3. Transfer spheroids gently from microwell plate to ClinoReactor® using a wide-bore pipette tip. We recommend opening the ClinoReactor like a petri-dish to accomplish this.
4. Fill ClinoReactor® completely with 10 mL media through top port.
5. Ensure no air is left in cell chamber, close top port and sterilise using ethanol.
6. Place ClinoReactor® in ClinoStar®.

Media change and speed adjustment

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.

Figures and Data



References

[1] S. A. Langhans, "Three-Dimensional in Vitro Cell Culture Models in Drug Discovery and Drug Repositioning," Frontiers in Pharmacology, vol. 9, 2018, [Online]. Available: https://www.frontiersin.org/article/10.3389/fphar.2018.00006

[2] K. Wrzesinski and S. Fey, "From 2D to 3D--a New Dimension for Modelling the Effect of Natural Products on Human Tissue," Current pharmaceutical design, vol. 21, no. 38, pp. 5605–5616, Oct. 2015, doi: 10.2174/1381612821666151002114227.