PRIMACYT
Cell Culture Technology

3D-Spheroid Culture of Cryopreserved Primary Hepatocytes

Updated 30.03.2021
Version 02

Recommended culture media and consumables

- 3D-HMM (3D-Hepatocyte Maintenance Medium): for serum-free culture of human, monkey, and dog hepatocytes
- 3D-HMM + 5% FBS
- 96-Well ULA round-bottom cell culture plates (e.g. Greiner Cat. No. 650 979, Corning Cat. No. 7007)

1. Seeding of hepatocytes

- Thaw cryopreserved hepatocytes according to supplier's manual or use fresh isolated primary hepatocytes
- After centrifugation according to suppliers manual, aspirate the supernatant
- Add 3D-HMM + 5% FBS to the cell pellet to get a cell density of 1-2 x 10^6 cells/ml
- Determine cell viability and live cell number with the trypan blue exclusion test in a counting chamber (do not use an automated cell counter)
- Adjust cell suspension for plating of 2500 cells/well in 70 µL/well
- Use 96-Well ULA round-bottom cell culture plates to seed the hepatocytes
- Spin the plate at 500 *x g*, 2 min, RT (room temperature) to bring the plated cells to the center (Fig. 1)

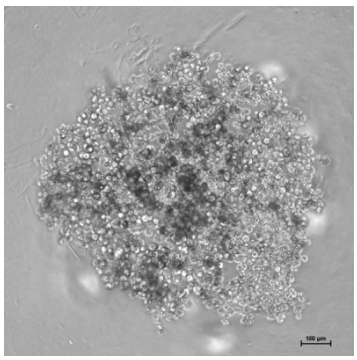

Fig. 1: Seeded and centered cells

- Let the cells rest and form spheroids for approx. 2-3 days at 37 °C and 5 % CO_2
- Perform a daily light-microscopic control. Handle the plate very carefully to avoid any disturbance of the spheroid formation.

2. Culture of hepatocyte spheroids

- After 2-3 days add 20 µl 3D-HMM to the inner wells and 30 µl to the outer wells. Spin the plates again at 500 *x g*, RT, for 1 min
- Let the cells rest for another 2-3 days at 37 °C and 5 % CO_2 until the cells achieve a nearly well-shaped form as shown in figure 2
- ***Note 1:*** *Spheroids from fresh hepatocytes often need more time to form. The addition of 20 µl/30 µl medium at a later time point (e.g. at day 5) is possible. A complete medium change should be performed no later than at day 7.*

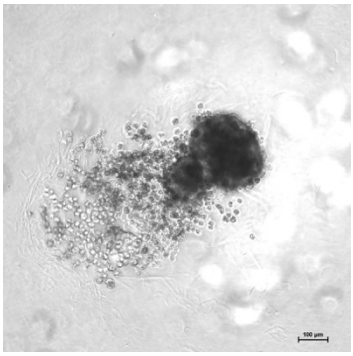

Fig. 2: Intact spheroids should be formed before the medium is completely changed for the first time

- Change whole medium by slowly aspirating the old medium with a pipet and add 70 µl of fresh 3D-HMM
- ***Note 2:*** *the spheroid will most certainly float around the well, while pipetting. Try not to aspirate it. If the spheroid is aspirated, make sure to release it slowly into the well again before you continue. A dark background (e.g. blue) makes it easier to see the spheroids.*
- ***Note 3:*** *Changing the whole medium in one 96-well plate will take 30-60 min depending on your experience.*
- Culture the spheroids another 1-2 days until the cells formed one well-shaped spheroid per well (figure 3). At this state individual test-assays may be performed.

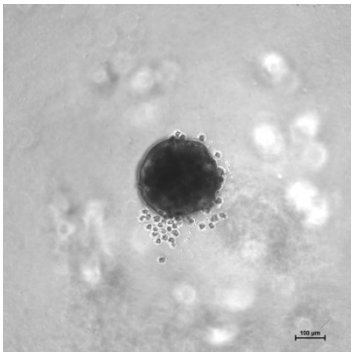

Fig. 3: Finished hepatocyte spheroid