Imaging Analysis of 3D Culture of Human Hepatoma Cells

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Abstract

3D culture of hepatocytes in extra cellular matrix forms polarized spheroid or organoid structures. Clear differences are seen in the cellular morphology and the localization and distribution of cell markers between monolayer and organoid cultures. Furthermore, hepatocyte spheroids or organoids can adopt either a proliferative or a metastatic state depending on the culture conditions. The long-term organoid culture of murine and human primary hepatocytes method has been described before. We used human hepatoma cells HepG2 and mouse hepatocyte AML-12 cells to culture 3D spheroids. Cells were maintained in conventional 2D culture and transferred to spheroid culture condition. AML-12 cells well formed spheroids on day 3 but lost the 3D spheroid morphology on day 7. Morphology of spheroids were examined by nuclear staining with hematoxylin. We found that 3D spheroid formed consistently similar size and shape in low-attachment petri dish, and the method delivered sufficient number of spheroids for experimental set up such as mitochondrial respiration (Seahorse) measurement, hepatocyte toxicity and hepatic stress response studies in future.

3D Low Attachment Method

In addition to hanging drop method, AML-12 cells in growth culture medium were cultured in low attachment petri dishes which we expected that cell-attachment to the dish was prevented, and spheroid formation was facilitated.

Objective

The objective of this study is to define the suitable method for spheroid culture of mouse and human hepatocytes in liver disease studies.

Background

The 3D tissue/cell culture is a technique utilized in embryology and microbiology to allow the growth that would otherwise be restricted by the 2D flat plane of culture dishes and also to minimize the surface area to volume ratio, slowing evaporation. Hanging drop culture to generate 3D spheroids was first adapted to culture nerve cells and also to minimize the surface area to volume ratio, slowing evaporation. The 3D tissue/cell culture is a technique utilized in embryology and microbiology to allow the growth that would otherwise be restricted by the 2D flat plane of culture dishes and also to minimize the surface area to volume ratio, slowing evaporation.

Method

Next, to determine the culture condition generating spheroids, AML-12 cells in cell culture dishes were detached and collected using TE buffer. Single cell suspension in low concentration was transferred to petri dishes. Cell growth continue from single cell to multiple cells and forms spheroids on day 3 (shown above). Spheroids tethered to the dish culture, but spheroids were detached from the dish and were rolling in the medium. Spheroids were collected and fixed in 1% neutral buffer formalin and nucleus was stained by hematoxylin (dense stained purple color).

Fig. 2. 3D spheroid formation in low-Attachment Petri Dishes. AML-12 cells in growth medium DMEM/F12 with supplements of fetal bovine serum, ITS, dexamethasone and penicillin/streptomycin were cultured in cell culture dishes. Cell were detached and collected using TE buffer. Single cell suspension in low concentration were transferred to petri dishes. Cell growth continue from single cell to multiple cells and forms spheroids on day 3 (shown above). Spheroids tethered to the dish culture, but spheroids were detached from the dish and were rolling in the medium. Spheroids were collected and fixed in 1% neutral buffer formalin and nucleus was stained by hematoxylin (dense stained purple color).

Spheroid size correlates with cell concentration

Next, to determine the culture condition generating spheroids, AML-12 cells in cell culture dishes were detached and collected using TE buffer, and different volume (0.5, 1, 3 ml) of single cell suspension was transferred to petri dishes to culture the cells at different cell concentration. As shown below different size of spheroids were formed on day 3.

Summary

• Spheroids formed in both hanging drop and low-attachment petri dish methods.
• Culturing in Low-attachment petri dish methods delivered sufficient number of spheroids for experimental set up such as mitochondrial respiration (Seahorse) measurement, hepatocyte toxicity and hepatic stress response studies during spheroid formation period day 3 to day 7.
• AML-12 spheroid created the spheroid microenvironment which changed the spheroid 3D to 2D formation in longer culture.

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