Co-Culturing of Primary Hepatocytes and Kupffer Cells in GrowDex® and DAPI/Phalloidin Staining of the Cell Spheroids

Written by Lauri Paasonen, Ph.D., UPM in collaboration with
Anne-Mari Moilanen, Ph.D., and Minna Komu, Ph.D., Admescope

GROWDEX® APPLICATION NOTE

INTRODUCTION

It is well known that three-dimensional (3D) culturing of hepatic cells resembles *in vivo* better than traditional monolayer cultures. Co-culturing of cells in 3D provides even more physiologically relevant approach to mimic *in vivo* tissue morphology and functionality due to intercellular communication between different cell types. Kupffer cells are specialized macrophages having an important role in the normal liver function.

Visualization of the cells during culture is an important factor, especially during the establishment of the model. Some 3D matrices can be problematic due to their inherent auto-fluorescence. However, GrowDex is not auto-fluorescent, therefore imaging labelled cells post staining is straightforward.

In this study we present a fluorescence staining methodology to determine cell viability and morphology of spheroids co-cultured from primary human hepatocytes and Kupffer cells in GrowDex. Confocal imaging was used to visualise both cell nuclei, stained with DAPI, and filamentous actin (F-actin), stained with Phalloidin, within the spheroid.

MATERIALS

- Human primary hepatocytes and Kupffer cells (Bioreclamation IVT)
- 96 well µ-plate (#89646 Ibidi)
- Hepatocyte cell culture medium (CP, Bioreclamation IVT) supplemented with 10 ng/ml hepatocyte growth factor (HGF, H5791, Sigma-Aldrich) and 20 ng/ml epidermal growth factor (EGF) (E9644, Sigma-Aldrich)
- Phosphate buffered saline (PBS)
- 4 % Paraformaldehyde (PFA) in PBS, 0.1 % Triton X-100 in PBS
- DAPI (28718-90-3, Sigma-Aldrich), Phalloidin (P1951, Sigma-Aldrich)

METHODS

1. Spheroids were cultured in GrowDex for 12 days according to the procedure presented in Application Note #7 - "Long-term 3D Culture of Human Primary Hepatocytes in GrowDex®" using Ibidi 96 well µ-plates.
    a) The ratio of primary hepatocytes to Kupffer cells was 2:1 (primary hepatoytes:Kupffer cells).
    b) 10 µl GrowDex containing 5,000 cells was dispensed into each Ibidi µ-well and 20 µl medium dispensed on top.
    c) The media was renewed every second or third day of culture.
    d) From the first medium change onwards, the culture volume was increased to 40 µl and only half of the medium (20 µl) replaced to avoid disruption of the GrowDex/cell layer.

2. Staining of the cells:
    a) Culture medium was removed from the wells and cells fixed with 60 µl of 4 % PFA in PBS for 1 hour at room temperature.
    b) PFA was removed and cells made permeable by the addition of 60 µl of 0.1 % Triton X-100 in PBS and incubated for 30 min at room temperature.
    c) Triton X-100 was removed and cells stained with 60 µl DAPI, 10 mg/ml stock solution diluted 1:500, and Phalloidin-TRITC diluted 1:1000, followed by overnight incubation at +4 °C.

3. Images of the spheroids were captured using a Zeiss LSM780 confocal microscope
    a) Plan-Apochromat 20x/0.8 objective
    b) DAPI: excitation 405 nm, emission 410 nm-533 nm
    c) Phalloidin-TRITC: excitation 561 nm, emission 566 nm-670 nm

RESULTS

Primary hepatocytes and Kupffer cells were co-cultured in GrowDex for 12 days and shown to form spheroids. The spheroids were successfully visualised using a confocal microscope after DAPI/Phalloidin staining of the cell nuclei and filamentous actin (Fig. 1).

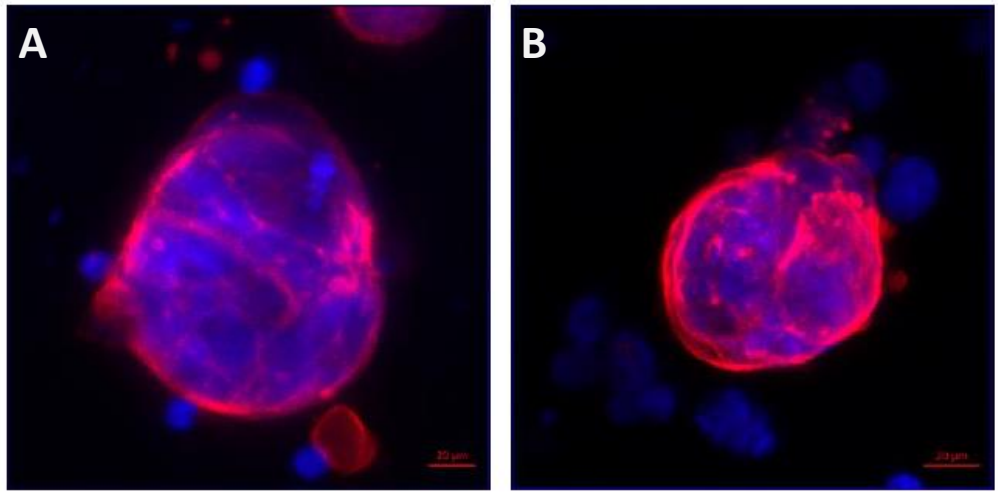

Figure 1. Primary hepatocyte/Kupffer co-culture (ratio 2:1). Spheroids stained with DAPI (blue) and Phalloidin (red) after 12 days culture in A) 0.8 % GrowDex, and B) 0.5 % GrowDex.

CONCLUSIONS

Relevant *in vitro* hepatic models are needed in drug development for e.g. hepatotoxicity or efficacy screening. Here we have presented a reliable method for 3D co-culturing of primary hepatocytes with Kupffer cells to form *in vitro* liver spheroids. In addition, a reliable and consistent procedure for staining liver cells with DAPI and Phalloidin, for visualisation of cell nuclei and actin filaments within spheroids cultured is also presented. Fluorescent dyes diffuse well and, as GrowDex is not auto-fluorescent, it provides an ideal 3D matrix for fluorescent staining and imaging of liver cell spheroids.