GrowDex®

Application Note 7

UPMBIOFORE BEYOND FOSSILS

Long-term 3D Culture of Human Primary Hepatocytes in GrowDex®

Lauri Paasonen, Ph.D.[1], Anne-Mari Moilanen, Ph.D.[2], Minna Komu, Ph.D.[2]

[1] UPM

[2] Admescope

INTRODUCTION

Long-term primary hepatocyte cultures are needed for repeated dose drug toxicity studies. It is well-known that hepatocytes in 2D culture lose their metabolic competence as well as viability around 1-2 weeks in culture. In this study 35 days culture of human primary hepatocytes on GrowDex is described. The viability of the cells was examined by measuring the lactate dehydrogenase (LDH) from cell culture medium collected at time points throughout the cultivation of the cells, and the cell functionality assessed by measuring the level of albumin secretion.

MATERIALS

- Human primary hepatocytes (LiverPool™, BioreclamationIVT)
- 96-well Ultra Low Attachment plate (Corning)
- Hepatocyte cell culture medium (CP, Bioreclamation IVT) supplemented with 10ng/ml hepatocyte growth factor (HGF) and 20 ng/ml epidermal growth factor (EGF)
- CytoTox-ONE LDH assay kit (Promega)
- Human Albumin ELISA kit (Abcam®)
- GrowDex 1.5 % (UPM)

METHODS

1. 0.5% GrowDex was prepared by diluting the stock hydrogel with hepatocyte cell culture medium supplemented with 10 ng/ml HGF and 20 ng/ml EGF.
2. 100 µl of 0.5% GrowDex was transferred to each well of a 96-well plate.
3. 50,000 cells were plated on top of 0.5% GrowDex in 100 µl volume
4. The microplate was incubated at 37°C, 5% CO2 for a total of 35 d (7d pre-culture to form spheroids + 28d spheroid culture). The medium was changed every second or every third day. Starting from the first medium change, medium volume was increased to 150 µl, and half of the amount was renewed to avoid the disruption of the sample.
5. Medium samples were taken from each microplate well and aliquots analysed for LDH and albumin release.
6. Viability of the cells was examined by analysing the LDH release during the culture.
7. Functionality of the cells was studied by analysing albumin secretion during the culture period.

admescope

UPMBIOMEDICALS

RESULTS

LDH release from the cells indicates the viability of primary hepatocytes throughout the experiment (Fig.1). Most of the cell death occurred during the 7 d pre-culture and during the following 7 d culture period (spheroid days 1-7). Albumin secretion was used as a biomarker for liver-like function of the primary hepatocytes. The albumin secretion observed throughout the experiment indicates good liver cell-like functionality (Fig.2).

Fig.1. The observed cytotoxicity measured as LDH leakage from the spheroids shows the cell viability during the 28 days spheroid culture. 7 day pre-culture = spheroid day 0.

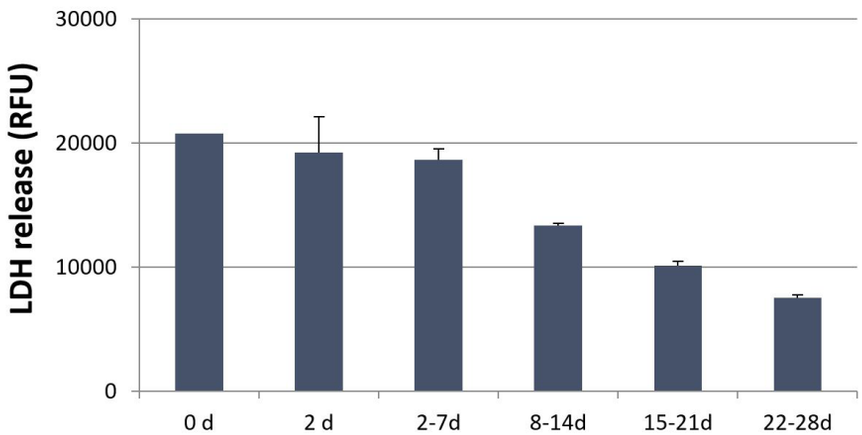
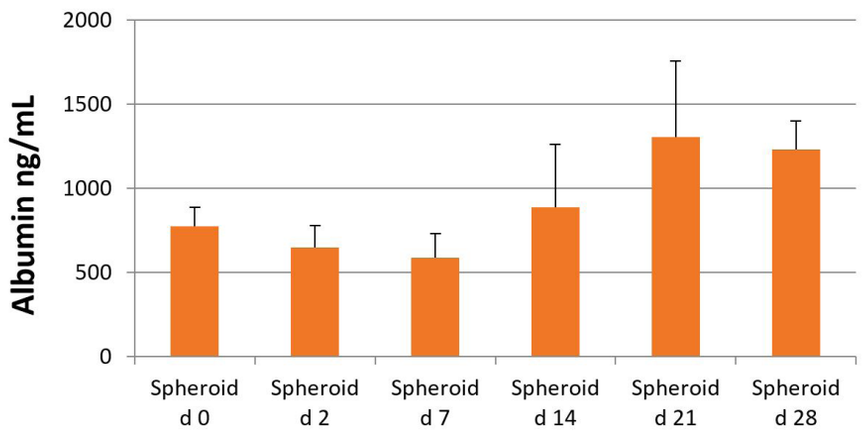


Fig. 2. Albumin secretion from primary hepatocytes during the 28 d spheroid culture (after 7d pre-culture) on GrowDex indicates the functionality of the cells. 7 d pre-culture = spheroid day 0.



CONCLUSIONS

It was shown that primary hepatocytes can be cultured in GrowDex for 35 days. The cells form 3D spheroid structures with good viability and liver cell-like functionality. Culturing of primary hepatocytes in GrowDex enables their use in extended applications, such as repeated dose toxicity studies.

You can order products online at: **www.upmbiomedicals.com/store**.
Or contact us at **biomedicals.sales@upm.com** for a quotation or to place an order.

UPM

www.upm.com

UPM Biomedicals
Alvar Aallon katu 1
P.O. Box 380
00101 Helsinki, Finland
biomedicals@upm.com
www.upmbiomedicals.com