

Analysis of CYP3A4 enzyme activity for primary hepatocytes cultured in 3D

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INTRODUCTION

As the liver is the most important organ in terms of foreign substance metabolism, the stability of enzymatic activity of hepatocytes is crucial. This is also true and of vital importance for *in vitro* liver models. Traditional cell culture methods for primary hepatocytes are limited due to the viability of the cells in a 2D format preventing extended culturing times. The use of a 3D culture model for primary hepatocytes, such as GrowDex[®], could enable cell viability to be extended and enable long-term *in vitro* experiments to be performed. In this study we measure CYP3A4 activity, the most common drug metabolizing enzyme in the liver, as a guide to cell functionality.

MATERIALS

- Human primary hepatocytes on GrowDex (LiverPool™, Bioreclamation IVT)
- KHB medium (Bioreclamation IVT)
- CYP3A4 specific substrate: testosterone
- GrowDex 1.5 % (UPM)

METHOD

1. Human primary hepatocytes were cultured on GrowDex as described in Application Note 007 "Long-term 3D Culture of Human Primary Hepatocytes in GrowDex"
2. Medium was removed from wells and replaced with 100µl KHB medium for 30 min
3. KHB medium was replaced with 100 µM testosterone in 100 µl KHB medium and incubated at 37 °C for 4 hours.
4. 50 µl of medium was removed, mixed with ice-cold acetonitrile and stored at -80 °C.
5. Samples were analyzed with mass spectrometry (Waters AcquityUPLC + Waters Xevo TQ-S triple quadrupole MS / Acquity HSS C18 (2.1 ×50 mm, 1.8 µm) + precolumn filter).

RESULTS

The metabolic activity of CYP3A4 in primary hepatocytes was tested by analysing the testosterone-6β-hydroxylation. The LC-MS analysis of testosterone hydroxylation indicates that the cells grown on GrowDex hydrogel stay enzymatically active over the 35 days culture days (7 days pre-culture + 28 days spheroid culture) (Fig.1). Enzyme activity in 2D cell culture at day 7 was set to 100%.

RESULTS CONTD

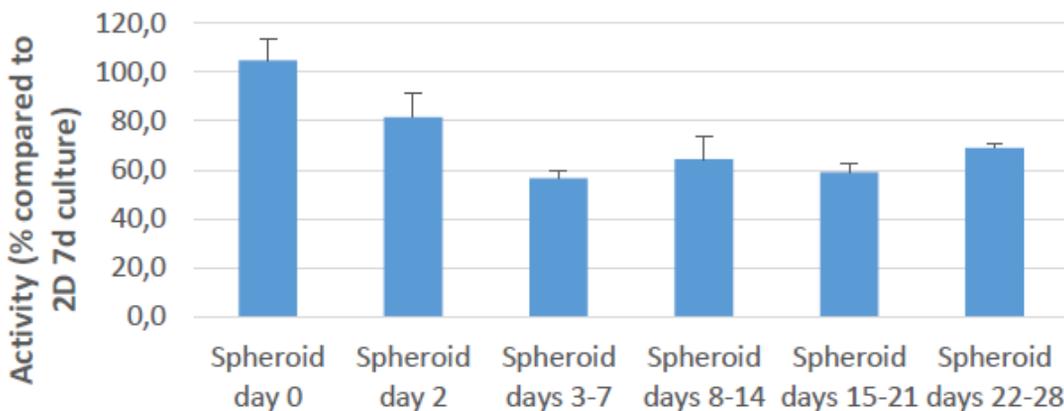


Fig. 1. CYP3A4 metabolic activity of primary hepatocytes during the 28 days 3D culture (after 7 days pre-culture) on GrowDex.

CONCLUSIONS

Primary hepatocytes retained their CYP3A4 activity during the 35 days culture on GrowDex shown by 6β-hydroxylation of testosterone. Long-term viability and enzymatic activity of primary hepatocytes enables their use in various applications, such as repeated dose drug toxicity studies.