Primary Göttingen Minipig hepatocytes for biomedical research and pharmaceutical development.

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Overview

Hepatocytes are the most prominent cells of the liver. They synthesize albumin, produce urea and eliminate toxic substances from the blood. In this biotransformation process transporter proteins (influx and efflux transporter) and phase I+II reactions play a central role. Primary hepatocytes are perfectly suited for in vitro metabolism and toxicity studies prior to preclinical tests. Minipig hepatocytes are isolated from livers obtained from male or female Göttingen Minipigs and are available fresh in suspensions or in various culture formats (6, 12, 24 and 96well) as well as cryopreserved cells.

Background

The liver is the main organ responsible for drug metabolism and detoxification. The parenchymal cells called hepatocytes, the most prominent cell type in the liver, are responsible for glucose homeostasis, albumin synthesis and urea production as well as for biotransformation. *In vivo*, other liver cell types (e.g. Kupffer cells, endothelial cells, Ito cells) interact *with the hepatocytes and* play a significant role by releasing of signal molecules, thereby contributing to growth, regeneration and immune response. Primary mammalian hepatocytes from various species are a very important tool for *in-vitro* prediction of uptake, metabolism and release of exogenous and endogenous substances as well as for toxicity and drug-drug-interaction *in vivo*.

Therefore, these cells are considered as valuable model systems for *in-vitro* metabolism and toxicity studies prior to species selection for preclinical tests. Hepatic CYP P450 enzymes show a high degree of similarity between human and pig, making the minipig a useful non-rodent model in general toxicity and safety pharmacology studies (1).

Göttingen Minipig hepatocytes

PRIMACYT has extensive practical experience in the isolation of primary hepatocytes from a large variety of species including Ellegaard Göttingen Minipig. The liver tissue is obtained from Ellegaard Göttingen Minipigs A/S (Dalmose, Denmark). The cells are available fresh as suspension and in various culture formats on collagen-coated plates (Figure 1) as well as cryopreserved (Figure 2). These primary cultures have the typical polygonal shape of hepatocytes and a high confluency allowing optimal cell-cell contacts. Studies on metabolism and toxicity can directly be performed at customer's site as well as at PRIMACYT

(GLP, Non-GLP). Hepatocytes are cultured under serum-free conditions in the chemically defined medium HHMM (Human Hepatocytes Maintenance Medium) (2). Hepatocyte specific morphology is maintained and cytochrome P450 protein activities are inducible under these conditions for at least 7 days in culture. Among their use in metabolism studies performed with suspension cultures, we have already used plated Göttingen Minipig hepatocytes in GLP studies for metabolic profiling in comparison to primary human hepatocytes.

Fresh isolated Göttingen Minipig hepatocytes for use in suspension at customer's site are stored in cold preservation solution and are shipped on crushed ice. The viability remains high until the day after isolation (81 +/-6 % after isolation vs. 78 +/- 7% on day 1, n=6) and makes these cells suitable for use in suspension assays for drug metabolism.

Göttingen Minipig hepatocytes at day 3 of culture in 24well plates cultured with HHMM (Fig. 1: fresh Minipig hepatocytes, Fig 2: cryopreserved Minipig hepatocytes).

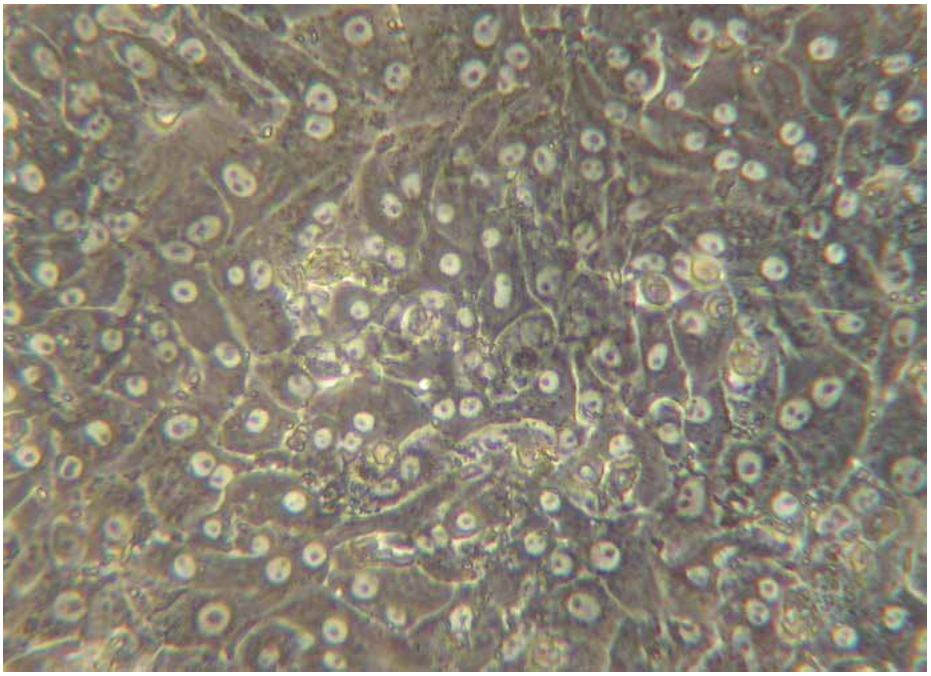

Figure 1: Phase contrast image of fresh isolated Göttingen Minipig hepatocytes on day 4 in culture.

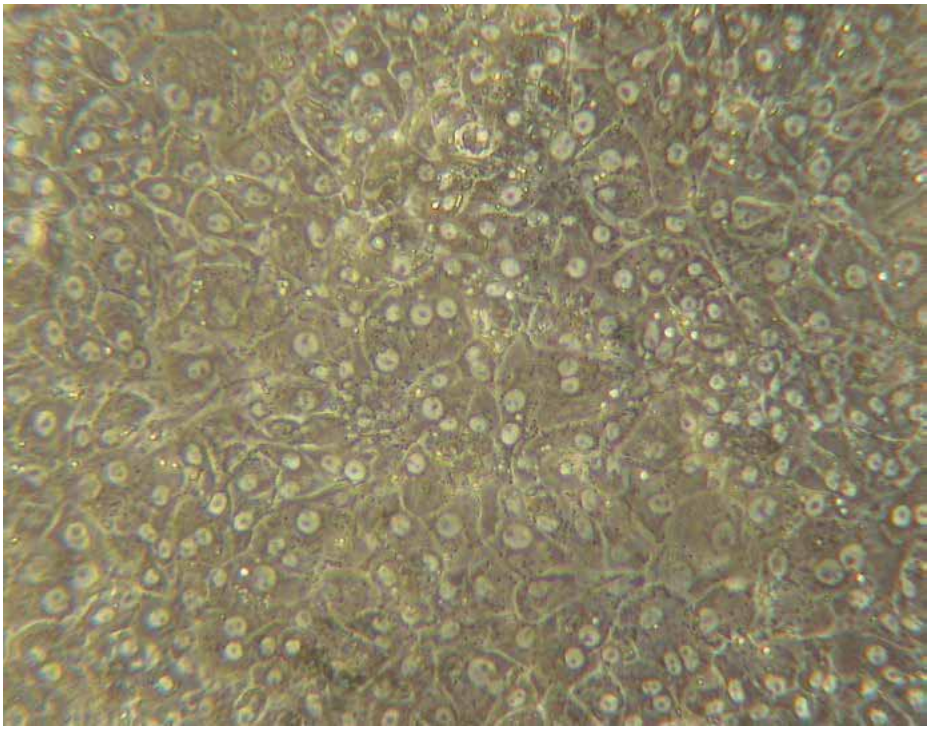

Figure 2: Phase contrast image of cryopreserved Göttingen Minipig hepatocytes on day 3 in culture.

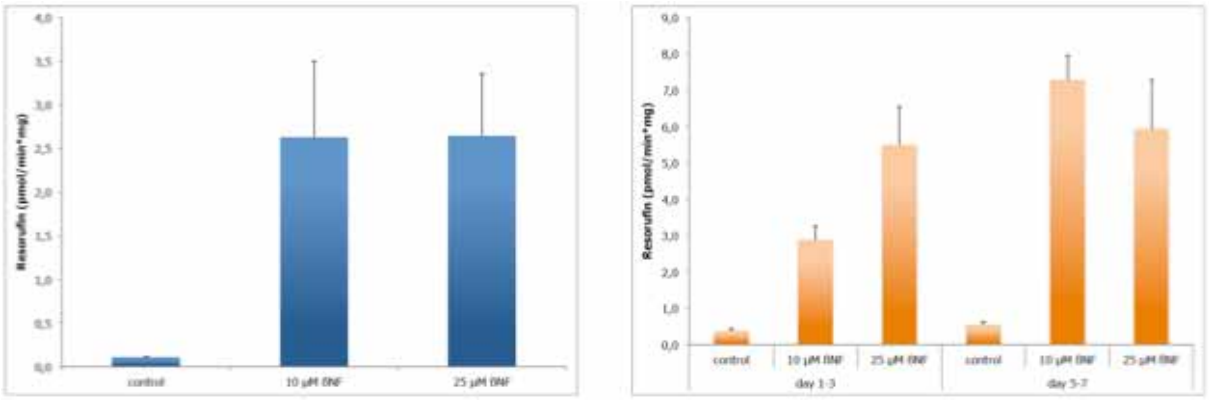

Fig 3: Induction of Ethoxyresorufin-O-deethylase (EROD) activity by ß-Naphthoflavone (ß-NF) for 48 h in Göttingen Minipig hepatocytes cultured with HHMM (left: fresh hepatocytes, induction day 3-5; right: cryopreserved hepatocytes, induction day 1-3 and day 5-7).

The potential of drugs to influence CYP P450 enzymes has to be evaluated as early as possible during drug development. Model inducers are applied as reference compounds in plated hepatocytes cultures. PRIMACYT uses β-Naphthoflavone (βNF) as model inducer for CYP1A2. The enzyme activity is inducible in fresh and cryopreserved Göttingen Minipig hepatocytes cultures (Figure 3). Both concentrations of βNF (10 µM and 25 µM) yielded to a 20- to 22-fold increase of EROD activity in cultures of fresh Minipig hepatocytes. Cryopreserved hepatocytes showed a slightly higher basal activity of CYP1A2 and a similar

response to βNF treatment in comparison to fresh isolated cells. The inducer βNF increased the CYP activity to 10- to 14-fold compared to untreated control cells.

Perspectives

In the last years, the Göttingen Minipig became a more preferred non-rodent species for regulatory toxicity testing since its organ anatomy, physiology and biochemistry is close to humans (1). Our results illustrate the use of primary hepatocytes isolated from Göttingen Minipig livers for preclinical studies prior to animal experiments. Thereby, they support the identification of drug-induced changes on liver-related processes. These applications in the 3R (reduce, replace, refine) context of alternatives to animal experiments may help to reduce the number of *in-vivo* studies in Minipigs and other species by collecting data on metabolism and toxicity beforehand.

More Product Details

Our fresh Göttingen Minipig hepatocytes are of highest quality. The level of quality insurance is identical with human hepatocytes. Typically we offer highest viability and functionality levels. In combination with appropriate cell morphology, our cell technology is one instrument for adequate *in vitro - in vivo* correlations for your purposes.

Characterization methods of fresh animal hepatocytes include the monitoring of liver specific functions, activities of drug transporters and the induction of cytochrome P450 activities. We also monitor and document cell morphology, attachment efficiency, monolayer confluency for platable cells and the overall appearance of the cells during culture.

Availability

Göttingen Minipig hepatocytes can be made available in suspension and in multi-well plates (6-, 12-, 24- and 96-well plate) on request. Please ask for availability or custom-made preparations.

Products in Suspension

Suspensions will be sent on crushed ice on the day of isolation and should be used on the day of receipt. Although hepatocytes in suspension are normally plateable within 20-24 hours after isolation, we do not guarantee the plateability of these cells, and recommend to order plated hepatocytes if this is the intended use. Included with each suspension order are MSDS and donor demographics.

Plated Products

Plated products will be sent at ambient temperature on day 1 after isolation and should be cultured at 37°C with 95% humidity and 5% CO2 atmosphere. Included with each order are following documents: datasheet (with microscopic images of cells prior to shipping), manual

for culture of primary hepatocytes, MSDS, and donor demographics.

Cells in plates require light protected culture at 37 °C and 5 % CO2 . However, conditions may be adjusted according to the body temperature of the individual species and with regard to the individual experimental design.

It is recommended to culture Minipig hepatocytes on collagen coated culture plates in HHMM (Human Hepatocyte Maintenance Medium), a serum-free culture medium containing Hepatocyte Growth Factor and Epidermal Growth Factor.

Final Note:
All personnel involved in potentially biologically hazardous activity share biosafety responsibility and must follow specified procedures, take appropriate training, act responsibly, and report incidents and hazardous circumstances. They should inform their supervisor of any personal condition such as illness, medications, pregnancy, or reduced immunity, which could make their work more hazardous to themselves and others.