Corning® Matrigel® Matrix-coated Transwell® Permeable Supports for Enhancing Hepatocyte Differentiation from Human Embryonic Stem Cells

Application Note

CORNING

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Introduction

Unforeseen adverse drug reactions in the liver is a major reason for the failure of drug candidates in clinical trials and for the after-market removal of drugs[1]. These issues arise as a result of drug metabolism occurring in the body. Primary human hepatocytes are considered the gold standard model to identify potential drug-drug interactions and toxicity using *in vitro* assays; however, their limited availability and inter-individual variability constrain their routine use early in the drug development process[2]. In recent years there has been considerable interest in generating renewable sources of hepatocytes from stem cells for drug safety studies[3]. Hepatic differentiation is typically accomplished by plating stem cells on tissue culture plates coated with collagen or Corning Matrigel matrix and sequentially culturing the cells with cytokines and growth factors that mimic fetal liver development[4]. While stem cell-derived hepatocytes are a promising approach to address the limitations of primary cells, existing differentiation protocols typically produce hepatic progeny whose basal and induced expression levels of major CYP drug metabolism genes (e.g., CYP1A2, CYP2B6, and CYP3A4) and key hepatic transcription factors is lower than what is observed in side-by-side comparisons with primary hepatocytes[5]. CYP3A4 is responsible for the metabolism of 40% to 50% of drugs on the market[6]. There is a clear need to identify culture formats and media that will further enhance the maturation of stem cell-derived hepatocytes.

In the liver, hepatocytes are normally arranged in one to two cell thick cords separated by sinusoids containing endothelial cells, Kupffer cells, Ito cells, and blood. Because of this unique tissue architecture, hepatocytes are polarized cells with two membrane surfaces exposed to blood[7]. We reasoned that carrying out hepatic differentiation of stem cells on permeable membrane supports, that permits exposure to nutrients, cytokines, growth factors, etc. from two directions, may enhance the maturation process. To investigate this question, hepatocyte progenitors derived from TW6 embryonic stem cells were cultured on Transwell permeable supports coated with Matrigel matrix and the basal and induced CYP expression was assayed using Promega's P450 CellTiter-Glo® assay kits and compared to HepaRG™ cells. Our results demonstrate that the Transwell culture format enhances both the basal and pharmacologically induced expression of CYP1A2 and CYP3A4.

Material and Methods

The TW6 human pluripotent stem cells used in this study were kindly provided by Wannshin Chen (Industrial Technology Research Institute, Hsinchu, Taiwan). HepaRG and recommended medium for CYP induction assays were procured from Life Technologies. DMEM/F12 medium, RPMI medium, B-27 supplement, Insulin-selenium-transferrin, Transwell 0.4 µm PET membrane cell culture inserts (Corning Cat. No. CLS3450), Corning Hepatocyte Maintenance Medium (CHHM) (Corning Cat. No. 40-550-CV), and Corning Matrigel hESC qualified matrix (Corning Cat. No. 354277) were from Corning. Hepatocyte growth factor (HGF), fibroblast growth factor 1 (FGF1), fibroblast growth factor 4 (FGF4) were purchased from Peprotech.

Hepatic Differentiation

TW6 cells were routinely maintained on mouse embryonic fibroblast feeder layers. hESC colonies were mechanically dissected into small pieces and re-plated on 2% collagen-coated dishes. Hepatic differentiation was carried out as previously described[8]. Briefly, cells were cultured with RPMI medium containing 1X B27 and activin A (100 ng/mL) to induce endoderm differentiation followed by co-treatment with fibroblast growth factor 4 (FGF4, 10 ng/mL) and hepatocyte growth factor (HGF, 10 ng/mL) to commit endodermal precursors to the hepatocyte lineage (Figure 1). At day 8, hepatic progenitors were subcultured and seeded onto Tissue Culture (TC)-treated polystyrene plates or Transwell inserts each coated with Corning Matrigel matrix (Figure 2). The cells were further differentiated in DMEM medium containing HGF (20 ng/mL) for 5 days, followed by DMEM/F12 medium plus oncostatin M (OSM) and dexamethazone (DEX) from day 9 to day 18. At day 19, cultures were overlaid with Matrigel matrix (25 µg/well) and re-fed with CHHM containing proprietary nutrient and non-nutrient differentiation supplements for 7 days to further promote hepatocyte maturation. For CYP induction studies hepatocytes were subsequently maintained in CHMM for 3 days with or without inducers before assaying cytochrome P450 enzymatic activity.

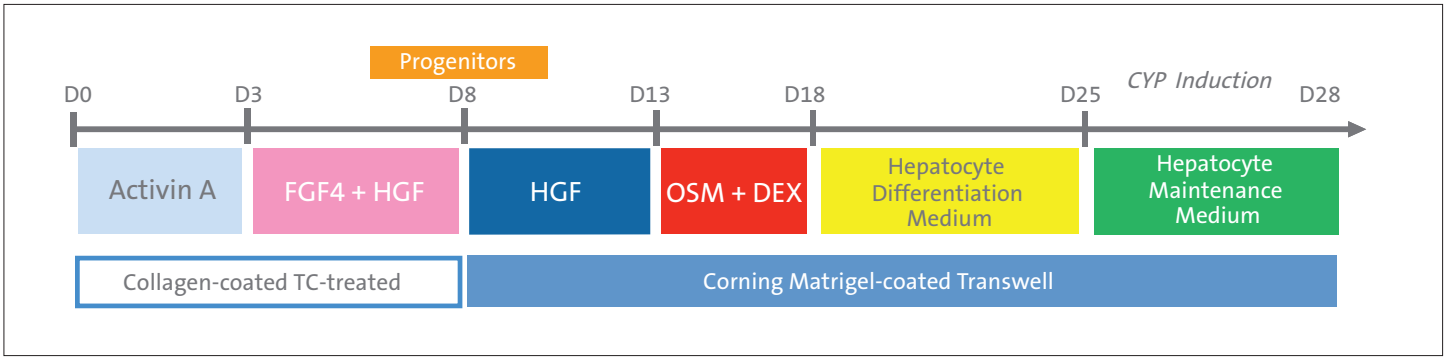

Figure 1. Hepatic differentiation protocol

CYP Induction Studies

For CYP induction studies, TW6-derived hepatocytes were cultured in CHHM containing 100 µM omeprazole or 10 µM rifampicin for 72 hours to induce CYP1A2 and CYP 3A4, respectively. Control cultures were maintained in CHMM containing 0.1% DMSO. CYP1A2 and CYP3A4 enzymatic activity was measured using P450 CellTiter-Glo® assay kits (Promega) according to the manufacturer's instructions. The activity of TW6-derived hepatocytes was compared to HepaRG™ cells cultured on collagen-coated TC-treated plates according to the manufacturer instructions.

Results and Discussion

The purpose of this study was to determine if differentiating hepatic progenitors on permeable supports would enhance their expression of CYP1A2 and CYP3A4. To investigate this question TW6 stem cell-derived hepatic progenitors were subcultured onto TC-treated dishes or Transwell® inserts coated with Corning® Matrigel® matrix on day 8. Hepatic progenitor differentiation was promoted by the sequential exposure to growth factors and cytokines which promote fetal liver development[4]. Hepatocyte maturation was further enhanced by culturing TW6 hepatocyte-like cells in Corning hepatocyte maintenance medium supplemented with nutrients and small molecules that enhanced the expression of liver-related transcription factors and decreased the expression of alpha-fetoprotein in 2D culture (data not shown).

In this study we observed that differentiating hepatic progenitors on Transwell supports in CHMM plus supplements further enhanced their maturation as measured by the enhanced expression of two key drug metabolism genes, CYP1A2 and CYP3A4 (Figures 3 and 4). Importantly, both the basal and induced expression of both CYP enzymes were elevated 2- to 3-fold in comparison to cells differentiated on Matrigel matrix-coated TC-treated plates. Moreover, CYP function of hepatocytes generated on Transwell inserts was similar to the activity of HepaRG cells maintained according to supplier's recommendations.

The results from this study clearly indicate that differentiating hepatic progenitors on permeable membrane supports enhances their subsequent maturation. How the Transwell culture format promotes hepatocyte differentiation is presently unclear. Other investigators have shown that co-culturing hepatocytes and endothelial cells on opposite sides of the Transwell membrane prolongs cell viability and hepatic function. The authors propose that their co-culture model mimics the *in vivo* liver sinusoid

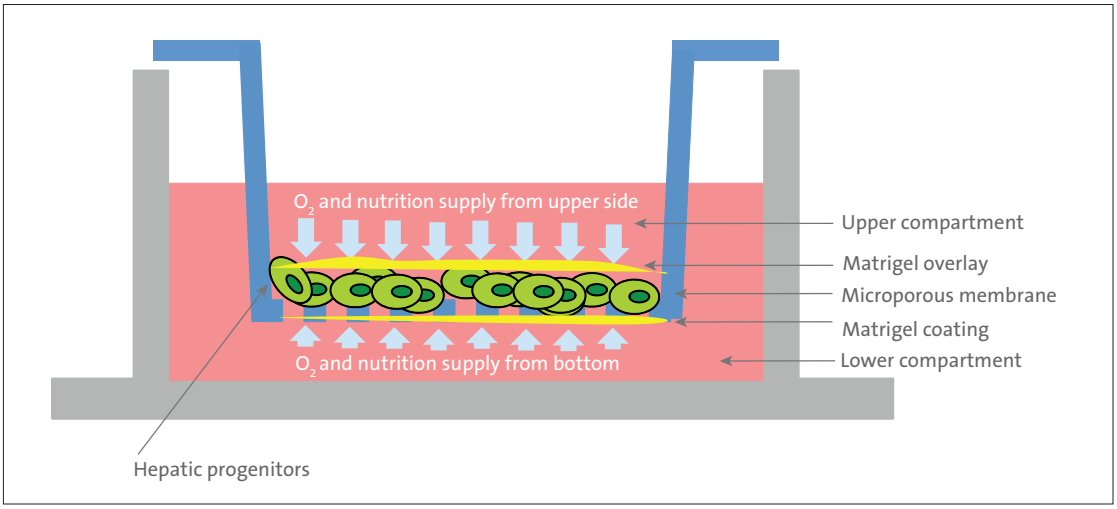

Figure 2. Differentiation of hepatic progenitors on Matrigel-coated Transwell inserts

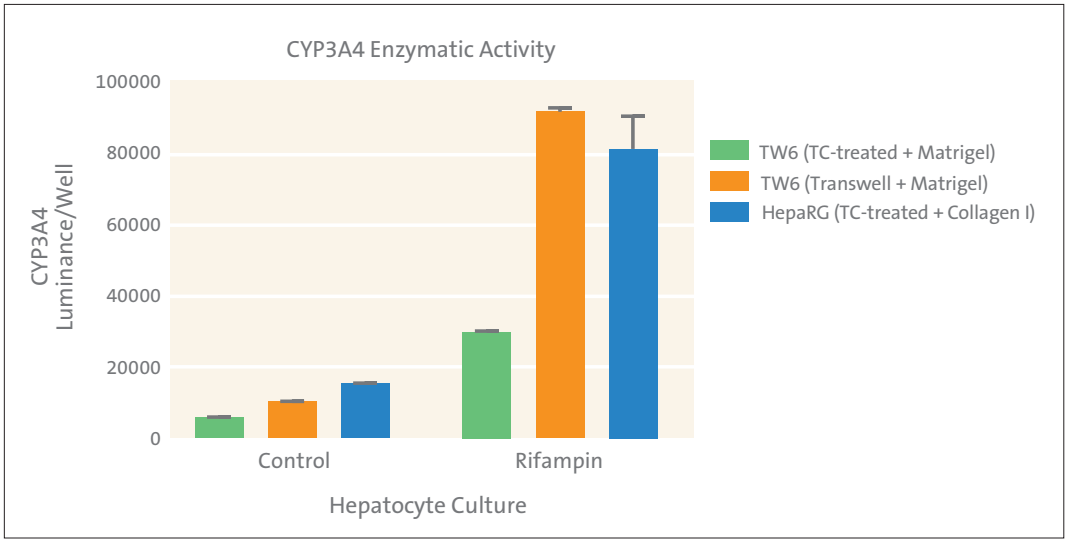

Figure 3. Differentiation of hepatic progenitors on Corning Matrigel matrix-coated Transwell inserts enhanced both basal and rifampin-induced CYP3A4 expression.

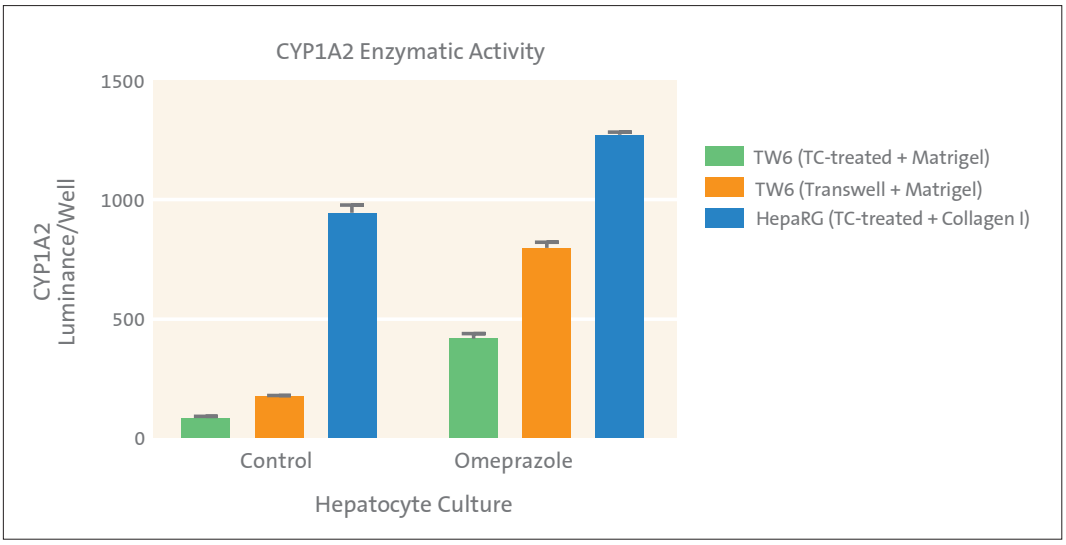

Figure 4. Differentiation of hepatic progenitors on Corning Matrigel matrix-coated Transwell inserts enhanced both basal and omeprazole-induced CYP1A2 expression.

architecture[9]. More recently, Dash, et al. reported that Transwell culture in combination with hemodynamic flow minimizes the rapid dedifferentiation of primary cultured rat hepatocytes and improves the expression of Phase I and Phase II drug metabolism genes[10]. In our study differentiated hepatocytes form a confluent monolayer on the Corning® Matrigel® matrix-coated Transwell® inserts, thus enabling exposure to nutrients and oxygen from two directions (Figure 2). This culture format is readily amenable for drug screening assays.

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