Corning® Matrigel® Matrix Thin-Coat and Corning Matrigel Matrix Overlay Improved CYP450 Activities in Human Cryopreserved Hepatocytes

Application Note 476

CORNING

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Abstract

The purpose of this study was to evaluate the effect of Corning Matrigel Matrix Thin-Coat and Corning Matrigel Matrix Overlay on the attachment, morphology, and CYP enzyme activities of human cryopreserved hepatocytes.

Corning Gentest™ Human CryoHepatocytes were tested for basal and induced CYP3A4 and CYP1A2 activities on Collagen I and Corning Matrigel Matrix thin-coat surface with or without a Corning Matrigel Matrix overlay. Changes in attachment and morphology were also examined. The results showed that the Corning Matrigel Matrix thin-coat surface significantly improved basal activities for both CYP3A4 and CYP1A2 compared to a conventional collagen I-coated surface; the Corning Matrigel Matrix thin-coat surface showed a lot-dependent effect towards induced activities for both CYP3A4 and CYP1A2 for tested lots. The Corning Matrigel Matrix overlay, in addition to the Corning Matrigel Matrix thin-coat plate, further improved basal CYP450 activities, however, the Corning Matrigel Matrix overlay did not change cell attachment. The Corning Matrigel Matrix thin-coat can maintain typical hepatocyte morphology for a longer time than the collagen I-coated surface. In conclusion, a culture condition combining Corning Matrigel Matrix thin-coat surface and Corning Matrigel Matrix overlay has a potential of maintaining stable long-term basal metabolic activities for human cryopreserved hepatocytes, facilitating their application in areas such as *in vitro* chronic toxicity assays.

Introduction

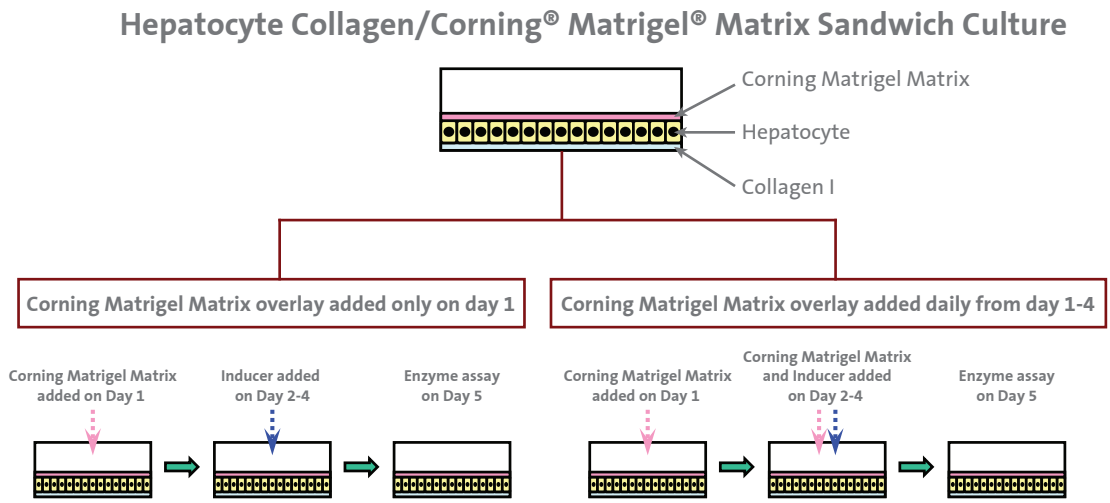
Primary human hepatocytes are considered the "Gold Standard" for drug metabolism studies. Induction in drug metabolizing enzymes in hepatocytes by test compounds can provide information for drug-drug interactions (DDI). Enzyme induction in hepatocytes takes a few days and both fresh and cryopreserved hepatocytes can be used for this purpose if they are both platable and inducible. Human cryopreserved hepatocytes often have limited application due to impaired cell attachment and enzyme activities which is caused by the cryopreservation process. Some cryopreserved lots have very low basal CYP450 activities, resulting in a very high fold of induction which could provide false DDI information. As cryopreserved hepatocytes have the benefits of being convenient, readily available, and able to provide multiple lots for comparison. It is however important to develop culture conditions that support cryopreserved hepatocyte recovery, hepatic morphology, and metabolizing activities, especially when long-term cell treatment is needed for certain applications such as *in vitro* chronic hepatotoxicity assays. It has been shown that extracellular matrix (ECM)-based growth substrata provide a physiological environment that maintains differentiation character and supports key cellular functions. Collagen I has traditionally been the substratum of choice for hepatocyte attachment and induction assays. Corning evaluated cell attachment, cell morphology and enzyme activities (especially basal activity which is an indicator of hepatocyte health) of cryopreserved hepatocytes using a Corning Matrigel Matrix thin-coat surface in the form of an overlay.

Methods

Culturing of Corning Gentest Human CryoHepatocytes

Corning Gentest Human CryoHepatocytes (Cat. Nos. 454550 and 454551) were thawed and purified using the Corning CryoHepatocyte Purification Kit (Cat. No. 454500). Purified hepatocytes were resuspended in ISOM's media containing 10% FBS at a concentration of $1.0x10^6$ cells/mL and seeded on 24 well plates (Corning BioCoat™ Collagen I-coated plate, Cat. No. 354408, Corning Matrigel Matrix Thin-Coat Plate, Cat. No. 354605) at a density of 400,000/well and incubated at 37°C with 5% CO_2. Corning Matrigel Matrix solution (0.25 mg/mL in Corning HepatoSTIM™ Medium) was added 6 hours later at 500 mL/well to form an overlay. In one set of experiments, Corning Matrigel Matrix overlay was added only on day 1; while in another set of experiments, Corning Matrigel Matrix overlay was added daily from day 1 to day 4.

The following graph illustrates the experimental set up for the ECM coating/overlay affect on hepatocyte application.



Hepatocyte CYP450 Induction Assay

Induction was conducted by adding inducers at 400 µL/well (20 mM Rifampicin for CYP3A4 and 20 mM ß-Naphthoflavone (ß-NF) for CYP1A2) daily from day 2 to day 4. Same amount of DMSO was added as vehicle control to obtain basal activities. On day 5, probe substrates (200 mM testosterone for CYP3A4 and 100 mM phenacetin for CYP1A2) were added and incubated with hepatocytes for 30 minutes (for 3A4) or 60 minutes (for 1A2). Supernatants were then collected into tubes containing stop solution and protein samples were collected by incubating cells with 1% SDS solution for 15 minutes. Metabolite samples were analyzed by HPLC and protein concentration was performed by Lowry assay.

Data analysis

The enzyme activity was expressed as pmol/mg protein/min. Each data point represents mean of 3 wells.

Results

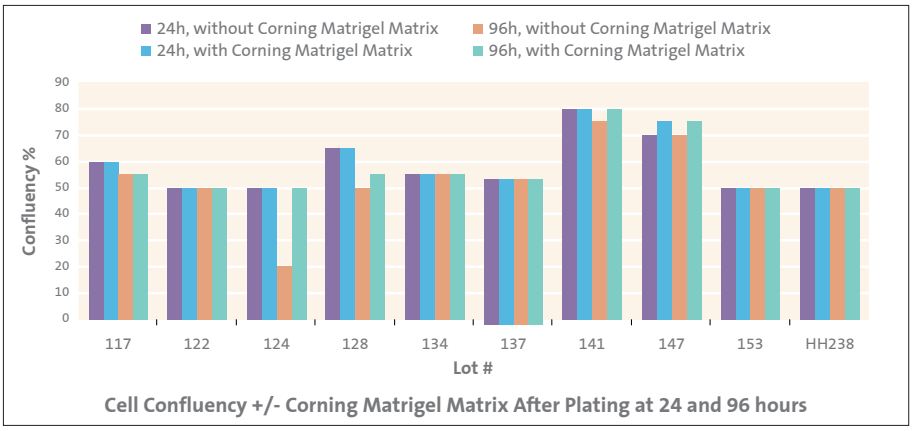


Figure 1. Effect of Corning Matrigel Matrix Overlay on Hepatocyte Attachment

Attachment of cryopreserved hepatocytes is not changed by the Corning Matrigel Matrix overlay.

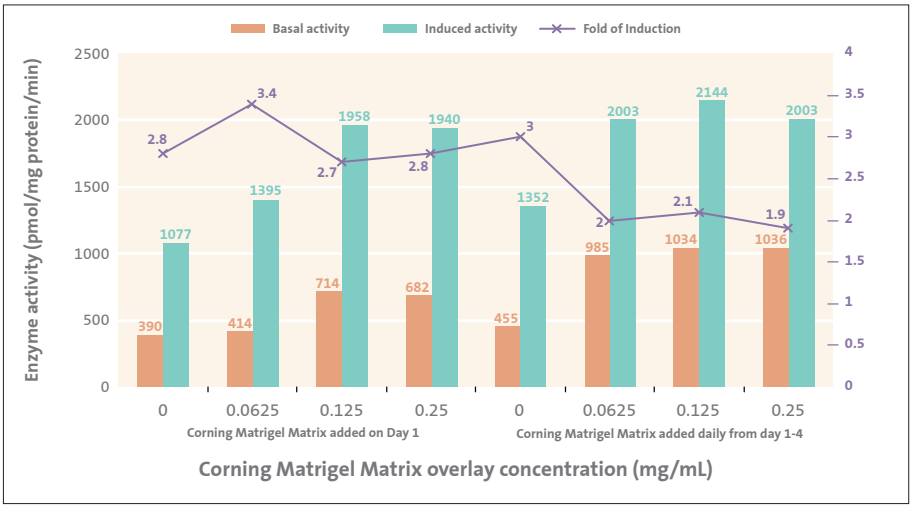


Figure 2. Optimization of Corning Matrigel Matrix Overlay for CYP3A4 Activities

Corning Matrigel Matrix was added at different concentrations and different frequencies to determine the optimum overlay format. It shows that the Corning Matrigel Matrix overlay significantly improved CYP3A4 basal activities in a concentration-dependent style up to 0.125 mg/mL. Induced activity was also increased at a lesser degree. No significant difference was observed with the frequency of adding Corning Matrigel Matrix.

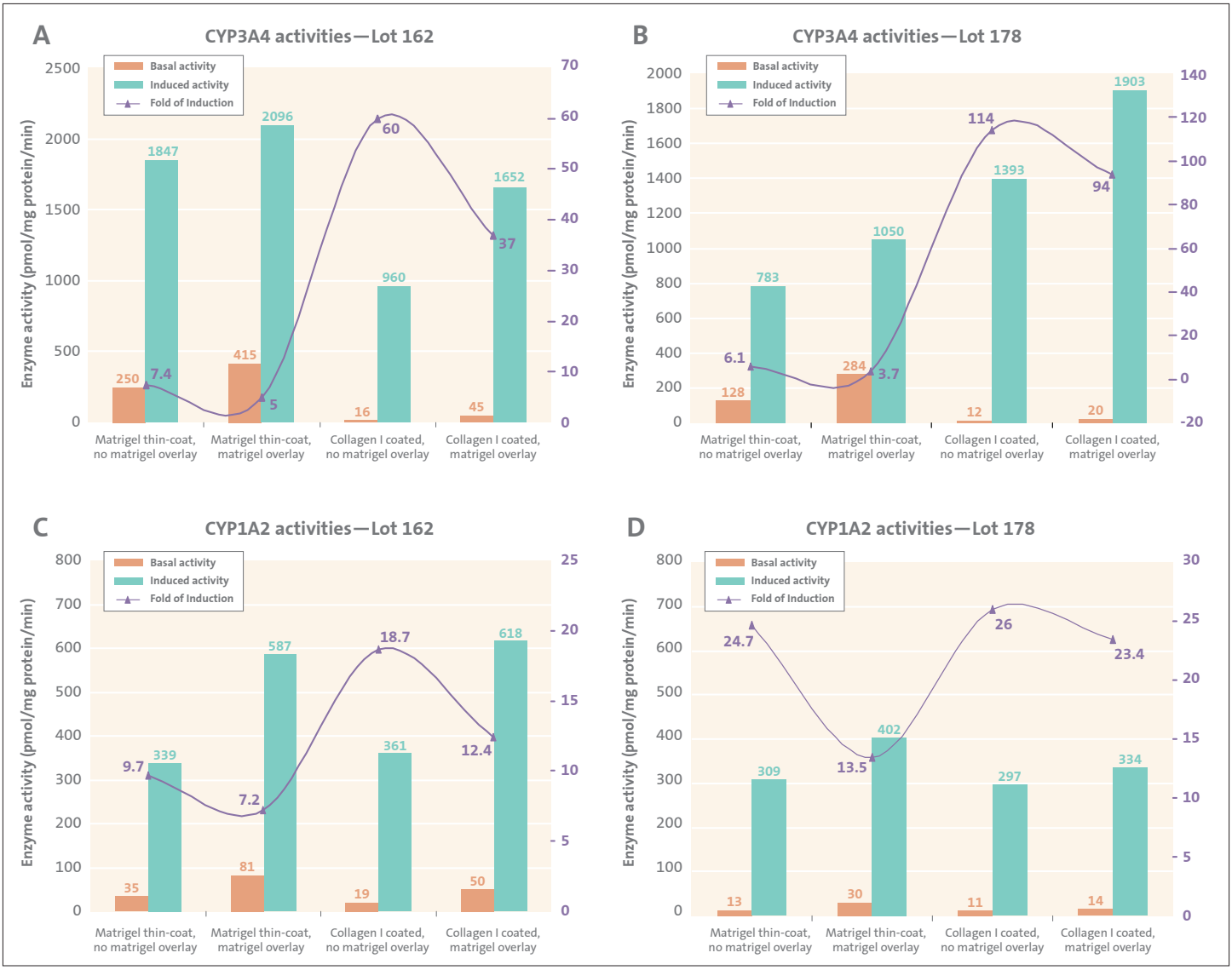


Figure 3. Corning® Matrigel® Matrix Thin-Coat Improves CYP450 Basal Activity

CYP3A4 basal activities in (A) lot 162 and (B) lot 178; CYP1A2 basal activities in (C) lot 162 and (D) lot 178.

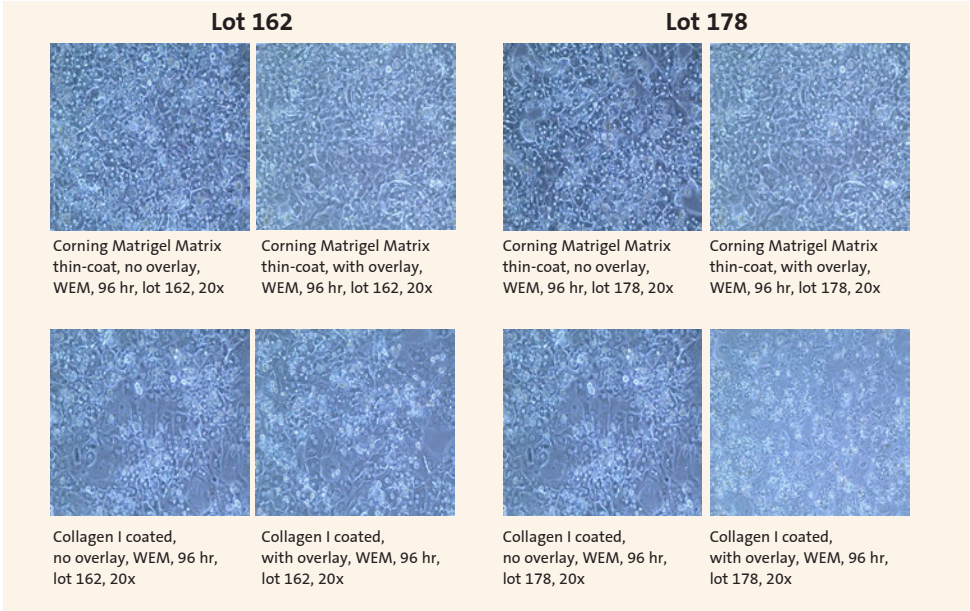


Figure 4. Corning Matrigel Matrix Thin-Coat Improves Cryopreserved Hepatocyte Monolayer Morphology

Summary and Conclusions

- Corning® Matrigel® Matrix thin-coat significantly improved basal activities for both CYP3A4 and CYP1A2 compared to conventional collagen I-coated surfaces.
- Corning Matrigel Matrix thin-coat increased induced CYP3A4 activity for lot 162 with no significant difference observed for lot 178 and CYP1A2, suggesting a lot-dependent effect on induced activity of cryopreserved hepatocytes.
- Corning Matrigel Matrix overlay, in addition to Corning Matrigel Matrix thin-coat substrate, further improved basal CYP450 activities; but it did not change attachment.
- Corning Matrigel Matrix thin-coat maintained hepatocyte morphology for a longer time than the collagen I surface.
- A culture condition combining Corning Matrigel Matrix thin-coat surface and Corning Matrigel Matrix overlay has a potential of maintaining stable long-term basal metabolic activities for cryopreserved human hepatocytes, facilitating the application in areas such as *in vitro* chronic toxicity assays.

References

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Corning acquired the BioCoat™, Gentest™, HepatoSTIM™, and Matrigel® brands.

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