Improved CYP450 Activity in Cryopreserved Hepatocytes with BD Matrigel™ Matrix Sandwich Cultures

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Topics

- Overview of hepatocyte products
- P450 induction (background information)
- Plateable cryopreserved hepatocytes for CYP Induction
- Hepatocyte Cultures with BD Matrigel™ Matrix Overlay
- Hepatocyte Cultures on BD Matrigel™ Matrix Thin-Coat Surface
Human Hepatocytes are Considered the "GOLD STANDARD" for DM Studies

- Hepatocytes are prepared from fresh human livers (organ donors)
- Hepatocytes are sold as:
  - Fresh cells on tissue culture plates
    - Induction studies
  - Fresh cells in suspension
    - Metabolic stability, transport studies, metabolite ID
  - Cryopreserved hepatocytes
    - Metabolic stability, transporter studies, metabolite ID
  - Cryopreserved hepatocytes that plate
    - Induction studies
Advantages

1. Can be plated and are inducible
   - CYP3A4, 1A2, 2B6, etc.
2. Convenient (use any time)
3. Availability – several lots
4. Easily make a “pool” of donors

Disadvantages

1. May have less attachment efficiency
2. Less robust to long-term culture
3. Not every lot inducible
P450 Induction

• “Induction” means to increase the cell content of the enzyme (protein)
  – Generally accomplished by transcriptional activation (increase the rate of mRNA synthesis)
  – Protein stabilization
  – Induced P450s and positive control inducers:
    • 3A4 (Rifampin)
    • 1A2 ( -Naphthoflavone)
    • 2C8/9/19 (Rifampin)
    • 2B6 (Phenobarbital)
    • 4A (clofibrate)
  – Fresh hepatocytes are the best in vitro system for induction studies (contain all necessary receptor pathways)
  – Cyropreserved hepatocytes are now available that can re-plate and be induced for CYPs
Drug-Drug Interactions – P450 Induction

- Drug that is a P450 inducer can increase the metabolism of other drugs or itself
  - Reduces drug AUC
  - Reduces drug efficacy
- Can lead to drug “tolerance” when drug can stimulate its own metabolism
- Induction can lead to generation of toxic metabolites
"Induction" is receptor mediated

**P450 Induction-Receptor Pathways**

- **Drug** → **DR-Cytosol** → **DR-Nucleus** → **Gene Transcription**

**Receptors**:
- AhR
- CAR
- PXR
- PPAR

**Enzyme Activity**

**mRNA** → **Protein**
CYP Induction Procedures (Hepatocyte Model)

Culture hepatocytes (2-3 days)

Treat with test drugs or positive inducers (3 days)

Harvest cells

Add substrates

mRNA

Microsomes

Enzyme activities

Northern blot RT/PCR

Western blot Enzyme activity

Fluorescence HPLC
Methods for Purification of Cryopreserved Human Hepatocytes

• **Percoll™-based Method**
  – Two-step Method with a 24% Isotonic Percoll gradient and rinse step
  – Percoll gradient removes dead cells increasing the viability of the cells (but cell yield is lower)

• **Non-Percoll Method**
  – One-Step Method is a simplified procedure
  – Include Percoll but initial viability is lower (but does not effect long-term viability or ability to induce)
  – Increased cell recovery
Procedure for Plating and Induction

- **Thawing**
  - Prepare cryopreserved human hepatocytes using BD Isotonic Percoll™ method

- **Plating**
  - Plate cells on 24-well collagen I-coated plate @ 400,000/well using ISOM’s Seeding Media
  - Incubate cells with 5% CO₂ at 37°C for 2-4 hours
  - Feed cells with BD™ Hepato-Stim media @ 400 µl/well and incubate with 5% CO₂ at 37°C for 2-4 hours

- **BD Matrigel™ Matrix Overlay**
  - Prepare BD Matrigel Matrix solution on ice
  - Add BD Matrigel Matrix @ 500 µl/well (0.25 mg/ml) with overlay media (WME)
  - Incubate the plates with 5% CO₂ at 37°C overnight

- **Induction**
  - Start 3-day CYP3A4 induction by adding 20 µM Rif and re-feed fresh inducing solution, each day from day 3-5
  - Perform enzyme assay on day 5
  - Analyze samples by HPLC and protein concentration performed by Lowry assay
• Cells will not be completely adherent after 2 hours, but they should be attached and be in the process of spreading

• After 24 hours, cells should be in cuboidal morphology

• Cryopreserved cells need to be used right away for induction assays
Inducible Cryopreserved Hepatocytes: Induction Data

Induction CYP3A4

Induction CYP1A2
Comparison of Fresh vs. Cryopreserved Hepatocytes on 3A4 Induction

![Graph showing comparison of Fresh vs. Cryo induction]

- **Fresh**
- **Cryo**
Effect of BD Matrigel™ Matrix Overlay on CYP450 Activities of Cryopreserved Human Hepatocytes
**BD Matrigel™ Matrix**

**A Reconstituted Basement Membrane**

Solubilized basement membrane preparation extracted from EHS mouse sarcoma

**Composition**

- **Laminin** ~ 60%
- **Collagen IV** ~ 30%
- **Entactin** ~ 8%
- **Heparan sulfate proteoglycan**
- **Growth factors (e.g., PDGF, EGF, TGF-)**
- **Matrix metalloproteinases**

![Diagram of basement membrane](image)
BD Matrigel™ Matrix Product Line

- BD BioCoat™ Matrigel Matrix Cellware
- BD BioCoat Matrigel Matrix Cellware–Thin Layer
- BD BioCoat Matrigel Matrix Cellware for Hepatocytes
- BD BioCoat Matrigel Matrix Plates for Embryonic Stem Cell Culture
- BD BioCoat Growth Factor Reduced Matrigel Matrix Cellware for Smooth Muscle Cells
- BD BioCoat Matrigel Matrix Cell Culture Inserts
- BD BioCoat Matrigel Invasion Chambers
- BD Matrigel Basement Membrane Matrix
Issues with Cryopreserved Hepatocytes for Induction

- Attached cells may not form stable monolayer
- Cells have good initial cell attachment (i.e. >70%) but low basal activity, resulting in abnormally high fold induction
  - Low basal activity can be a problem for assays with low sensitivity
Objectives

To determine if BD Matrigel™ Matrix can….

- Improve cell attachment and morphology
- Improve basal activity
- Improve induced activity
- Effect fold-induction
Hepatocyte Collagen/BD Matrigel Matrix Sandwich Culture

- BD Matrigel Matrix added on Day 1
- Inducer added on Day 2-4
- Enzyme assay on Day 5

BD Matrigel Matrix Overlay added only on Day 1

BD Matrigel Matrix Overlay added daily from Day 1-4

- BD Matrigel Matrix and Inducer added on Day 2-4
- Enzyme assay on Day 5
Attachment (%) at 24 and 96 Hours after Plating

- BD Matrigel™ Matrix overlay generally did not change cell attachment
BD Matrigel™ Matrix Overlay Maintained Cell Morphology of Human Cryopreserved Hepatocytes

- BD Matrigel Matrix overlay slightly improved cell morphology
Effect of BD Matrigel™ Matrix Overlay on CYP3A4 Activity

- BD Matrigel Matrix overlay increased both CYP3A4 basal activities and induced activities
- Fold induction was less effected (trend was a slight reduction in fold induction [basal increased more than induced activity])
- No significant improvement was seen when BD Matrigel Matrix was added on multiple days
Optimization of BD Matrigel™ Matrix Overlay for CYP3A4 Activity

- Increase in basal/induced CYP3A4 activities was BD Matrigel Matrix concentration-dependent up to 125 µg/ml
Summary of BD Matrigel™ Matrix Overlay Results

- BD Matrigel Matrix overlay did not change cell attachment efficiency
- BD Matrigel Matrix overlay improved basal and induced CYP3A4 activity
- BD Matrigel Matrix overlay improved cell morphology
Effect of BD Matrigel™ Matrix: BD Matrigel Matrix Sandwich Environment on Cryopreserved Hepatocyte Function
Objective

- Determine if BD Matrigel™ Matrix thin-coat surface alone, or in combination with BD Matrigel Matrix overlay, can improve hepatocyte basal or induced activity, fold-induction and cell morphology
ECM Coating/Overlay Effect on CYP450 Activities—Design of Experiments

Hepatocyte Collagen/BD Matrigel™ Matrix Sandwich culture

- Cultured on BD Matrigel Matrix thin-coat plate
  - No overlay
  - With BD Matrigel Matrix overlay

- Cultured on Collagen I plate
  - No overlay
  - With BD Matrigel Matrix overlay
Result: BD Matrigel™ Matrix Coating and Overlay Effect on CYP3A4 Activities of Human Cryopreserved Hepatocytes

- BD Matrigel Matrix thin-coat significantly improved basal CYP3A4 activity compared to collagen I surface. Fold induction is more “physiological” (consistent with in vivo results)
- BD Matrigel Matrix overlay further increased basal activity and induced activity
Result: BD Matrigel™ Matrix Coating and Overlay Effect on CYP1A2 Activities of Human Cryopreserved Hepatocytes

<table>
<thead>
<tr>
<th>Exp. Condition</th>
<th>Fold-Induction</th>
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<tbody>
<tr>
<td>Matrigel Thin-Coat Coated Plate</td>
<td>10</td>
</tr>
<tr>
<td>Matrigel Thin-Coat/+ Matrigel Overlay</td>
<td>7</td>
</tr>
<tr>
<td>Collagen I Coated Plate</td>
<td>19</td>
</tr>
<tr>
<td>Collagen I Coat/+ Matrigel Overlay</td>
<td>12</td>
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</tbody>
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On the collagen I coated surface, hepatocytes quickly spread and showed typical cuboidal morphology in less than 7 hours; while on the BD Matrigel Matrix surface, hepatocytes remained spherical and formed cell aggregates for at least 24 hours.
Effect of ECM Coating and Overlay on Cell Morphology at 96 hours

**Lot 162**
- BD Matrigel™ Matrix thin-coat, no overlay, 96 hr, lot 162, 20x
- BD Matrigel Matrix thin-coat, with overlay, 96 hr, lot 162, 20x
- Collagen I-coated, no overlay, 96 hr, lot 162, 20x
- Collagen I-coated, with overlay, 96 hr, lot 162, 20x

**Lot 178**
- BD Matrigel Matrix thin-coat, no overlay, 96 hr, lot 178, 20x
- BD Matrigel Matrix thin-coat, with overlay, 96 hr, lot 178, 20x
- Collagen I-coated, no overlay, 96 hr, lot 178, 20x
- Collagen I-coated, with overlay, 96 hr, lot 178, 20x

**Improved morphology by BD Matrigel™ Matrix thin-coat vs. Collagen I-coated surface**
Conclusions

• BD Matrigel™ Matrix thin-coat maintained hepatocyte morphology and 3D structure for a longer time than Collagen I-coated surface

• BD Matrigel Matrix “sandwich” environment produced more physiological fold-induction vs. collagen surface or BD Matrigel Matrix overlay/collagen sandwich

• Sandwich environment has the potential for maintaining longer term basal metabolic activities in cryopreserved human hepatocytes and may facilitate applications such as chronic tox studies
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