Effect of Matrigel Overlay and Matrigel Thin Coat on CYP450 Activities in Cryo Human Hepatocytes

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Topics

- Overview of Hepatocyte Products
- P450 Induction (Background Info)
- Plateable Cryopreserved Hepatocytes for CYP Induction
- Hepatocyte Cultures with BD Matrigel™ matrix Overlay
- Hepatocyte Cultures on BD Matrigel matrix Thin-Coat Surface

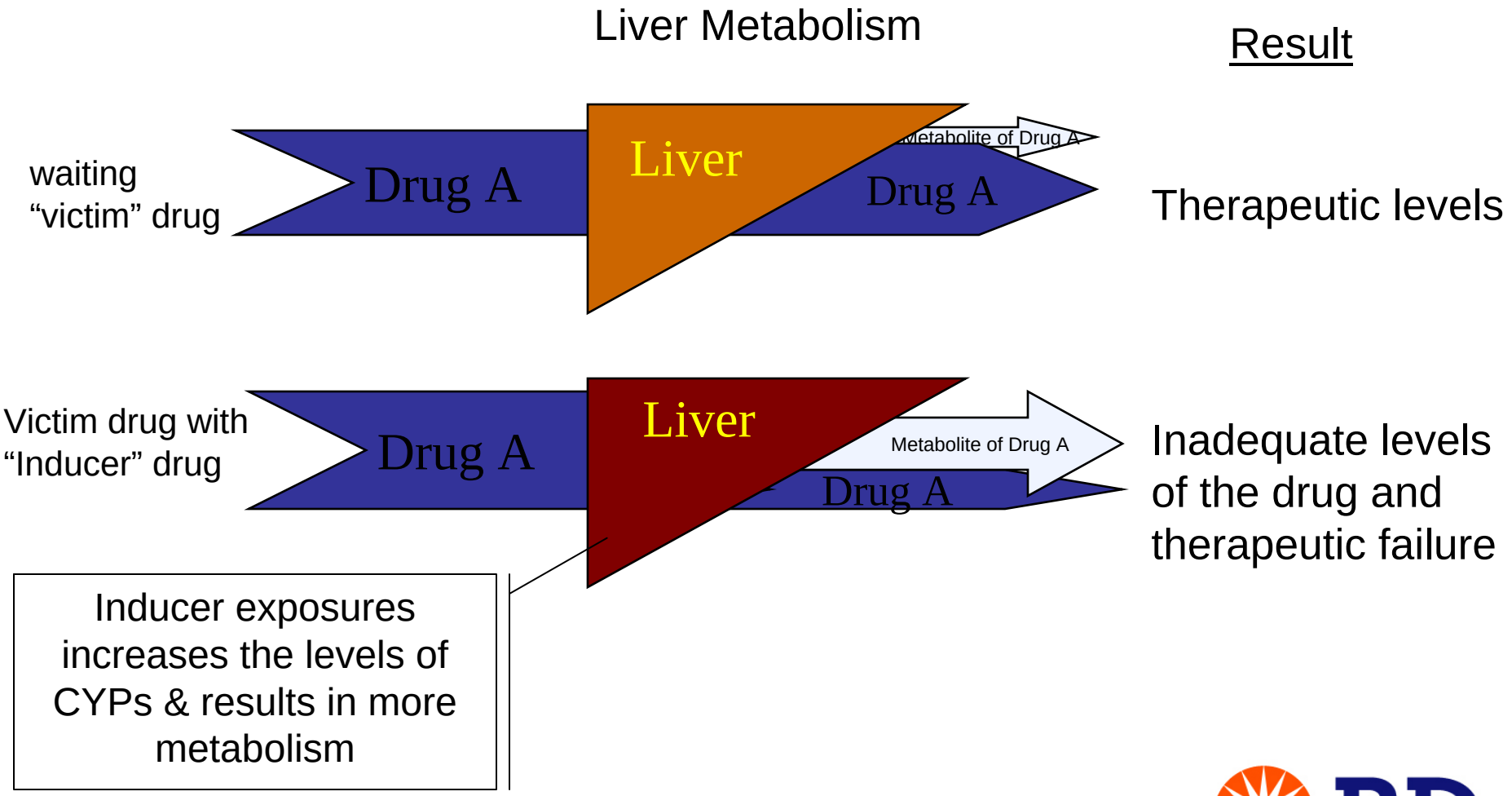
Human Hepatocytes Are Considered the "GOLD STANDARD" for DM Studies

- **Hepatocytes are prepared from fresh human livers (organ donors)**
- **Key Applications**
  - Metabolite profiling
  - Metabolic stability
  - Enzyme induction
  - Drug transport
  - Drug toxicity
- **Short term assays in suspension**
  - Metabolite profile, metabolic stability, drug transport, drug toxicity
- **Longer term assays in plated cells**

Drug-Drug Interactions - P450 Induction

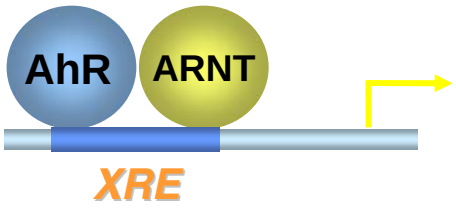
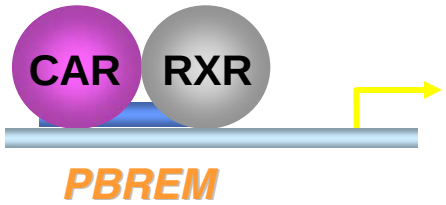
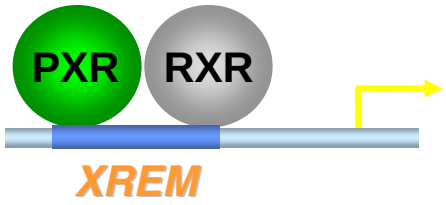
- Cytochrome P450 induction is an increase in the amount of protein and enzyme activity
  - More enzyme means more clearance and less drug in circulation
  - Represents another scenario for drug-drug interactions (opposite of what is seen with CYP inhibition)
- CYP Induction causes a reduction in the efficacy of co-administered drug.
- CYP induction can lead to drug “tolerance” when drug can stimulate its own metabolism
- CYP Induction can lead to generation of toxic metabolites
- This is a complex assay and is typically done later in lead optimization or in development

Induction-based Drug-drug Interaction



How Does It Work? - P450 Induction Receptor Pathways

Drug → DR-Cytosol → DR-Nucleus → Gene Transcription → mRNA → Protein → Enzyme Activity

Prototypical Inducer(s) (positive control)	Nuclear Receptors	Biomarkers
ß-naphthoflavone 3-Methylcholanthrene Omeprazole Lansoprazole	Aryl hydrocarbon receptor 	*CYP1A*, UGT1A1
Phenobarbital Phenytoin	Constitutive androstane receptor 	*CYP2B*, CYP3A, CYP2C, UGT1A1
Rifampin (human) PCN (rat)	Pregnane X receptor 	*CYP3A*, CYP2C, CYP2B, MDR1, MRP2, UGT1A1

P450 Induction Test System

- Receptor binding assay
- Gene-reporter constructs
- Hepatocyte-like cell line (e.g., HepaRG)
- 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

CryoHepatocytes

Advantage

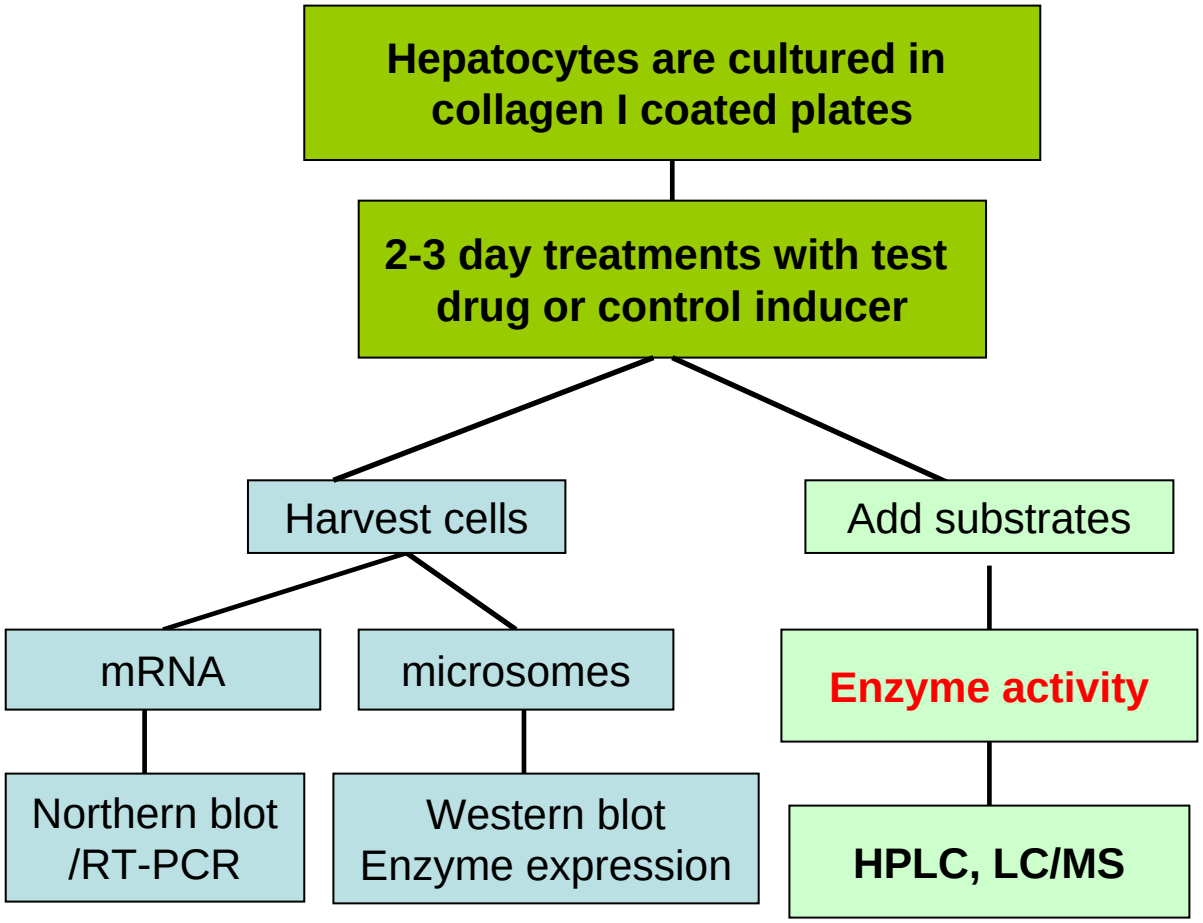
- Can be plated and are inducible
- Convenient
- Availability - several lots
- Easily make a “pool” of donors
- Gold standard for CYP induction studies
- Best *in vitro* to *in vivo* prediction for drug clearance

Disadvantage

- Less attachment efficiency
- Less robust to long-term culture
- Not every lot inducible

BD

P450 Induction Assays in Plated Hepatocytes

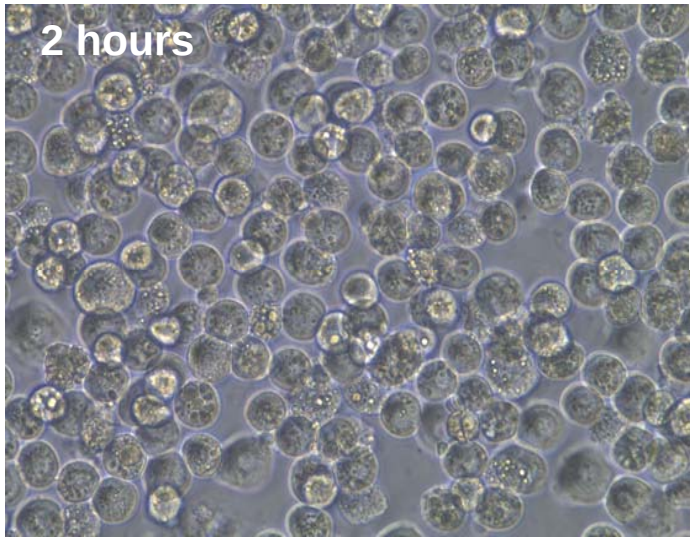


Multiple endpoints can be used

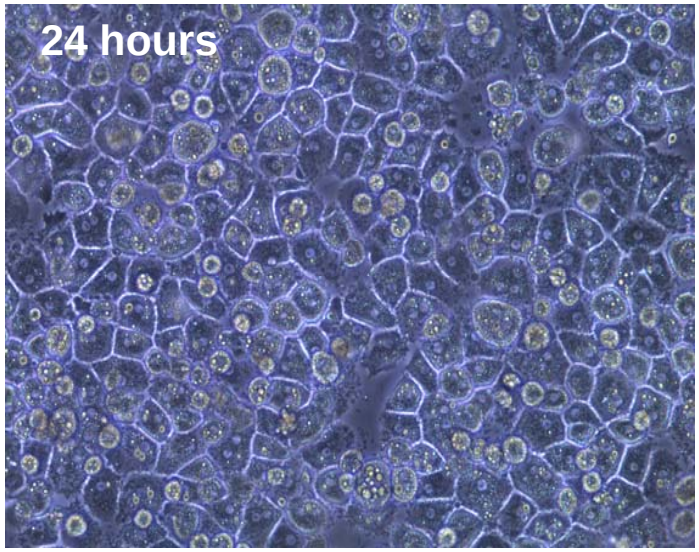
Procedure for Plating and Induction

- **Thawing**
  - Cryopreserverd human hepatocytes were thawed using BD hepatocyte recovery medium
- **Plating**
  - Plate cells on 24 well collagen I-coated plate @ 400,000 cells/well using ISOM's Seeding Media
  - Incubate cells with 5% CO_2 at 37^{o}C for 2-4 hours
  - Change media to BD™ Hepato-Stim media @ 400 µl/well and incubate with 5% CO_2 at 37^{o}C or add BD Matrigel™ matrix overlay
- **BD Matrigel Matrix Overlay**

Cell Attachment Post Thaw

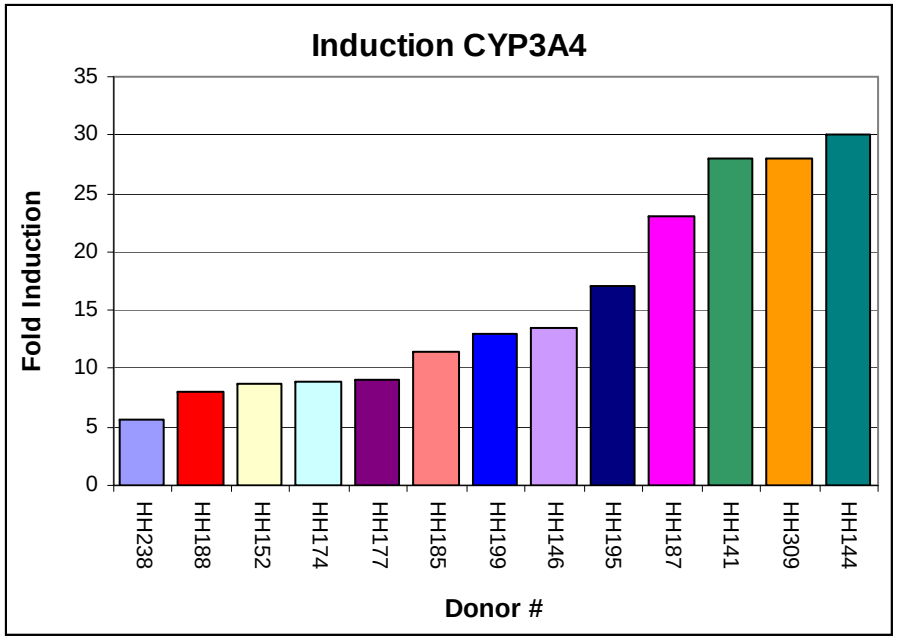


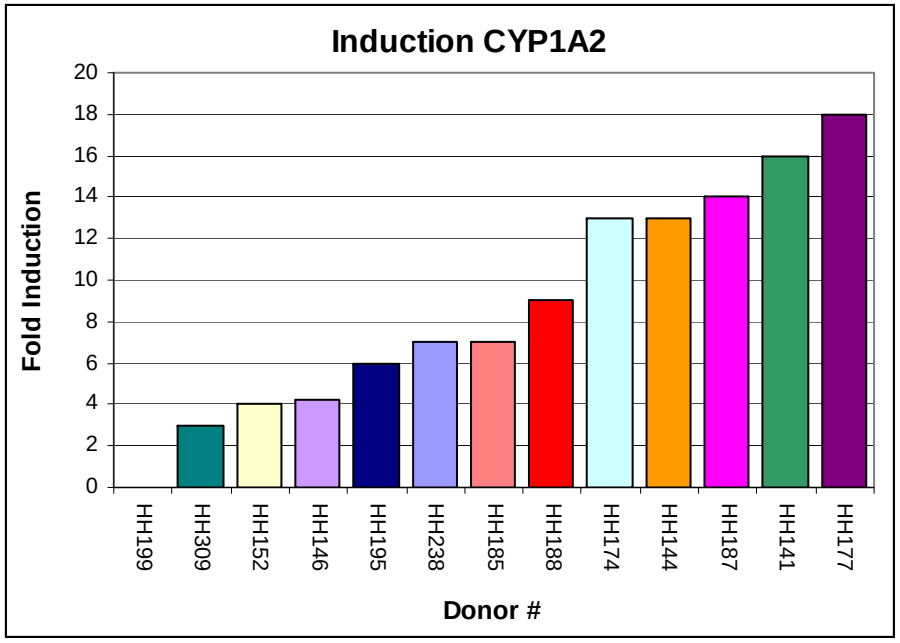
- Cells will not be completely adherent after 2 hours, but they should be attached and be in the process of spreading



- After 24 hours, cells should be in cuboidal morphology

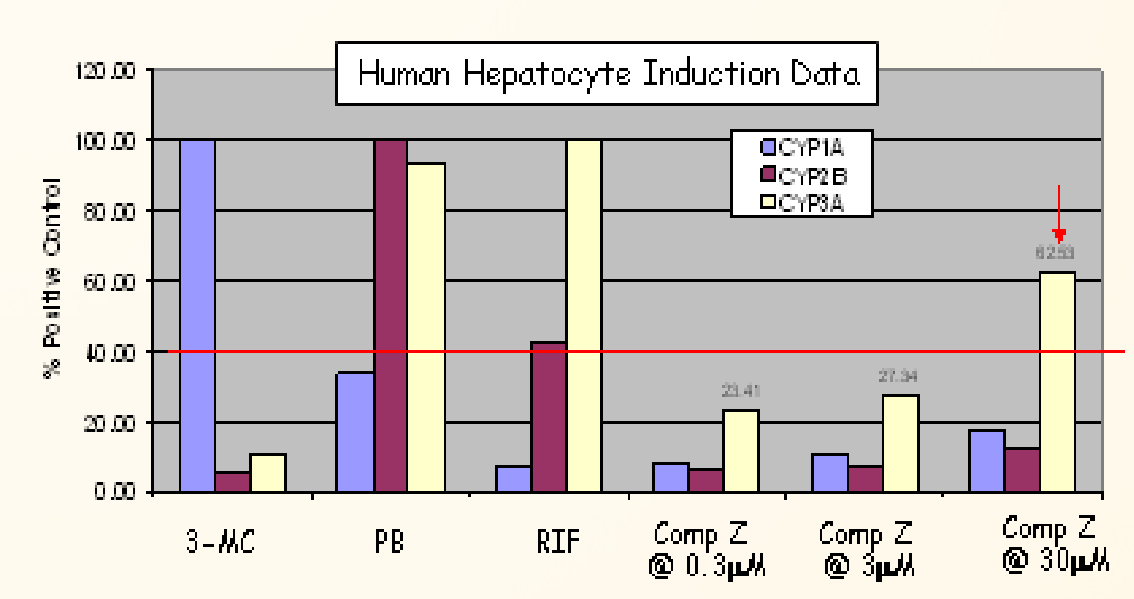
Inducible Cryopreserved Hepatocytes: Induction Data





Example Results

- The enzyme activity is normalized to the response of the positive control
- Compounds which give ≥40% of the positive control response are considered inducers



Keys to Success

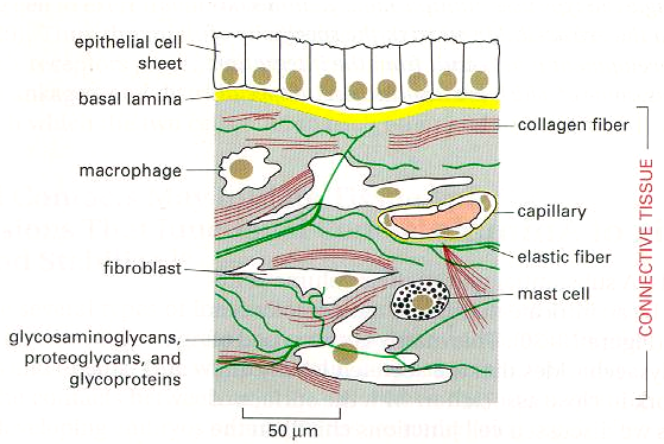
- Post thaw viability
  - Low post thaw viability may result in poor plating attachment
- Plating efficiency
  - Poor plating efficiency may skew (lower) enzyme activity
- Reasonable basal activity
  - Too low basal activity may cause large data variance
  - Too low basal activity may result in abnormally high fold induction

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

BD Matrigel Matrix Product Line

BD BioCoat™ Matrigel™ Matrix Cellware		
354432	BD BioCoat™ BD Matrigel™ Matrix Cellware	6-well plates
354503	BD BioCoat Matrigel Matrix Cellware	12-well plates
354433	BD BioCoat Matrigel Matrix Cellware	24-well plates
354508	BD BioCoat Matrigel Matrix Cellware	48-well plates
354460	BD BioCoat Matrigel Matrix Cellware	35 mm culture dishes
BD Matrigel™ Matrix—Thin Layer		
354603	BD BioCoat Matrigel Matrix Cellware—thin layer	6-well plates
354605	BD BioCoat Matrigel Matrix Cellware—thin layer	24-well plates
354607	BD BioCoat Matrigel Matrix Cellware—thin layer	96-well plates
354602	BD BioCoat Matrigel Matrix Cellware—thin layer	35 mm culture dishes
354601	BD BioCoat Matrigel Matrix Cellware—thin layer	60 mm culture dishes
354600	BD BioCoat Matrigel Matrix Cellware—thin layer	100 mm culture dishes
BD Matrigel™ Matrix for Hepatocytes		
354510	BD BioCoat Matrigel Matrix Cellware—for hepatocytes	6-well plates
Growth Factor Reduced Matrigel Matrix for Smooth Muscle Cells		
354635	BD BioCoat GFR Matrigel Matrix Cellware—for SMC	24-well plates
BD BioCoat™ Matrigel™ Matrix Plates for Embryonic Stem Cell Culture		
354671		BD BioCoat GFR Matrigel Matrix Cellware—for SMC
BD BioCoat™ Matrigel™ Matrix Cell Culture Inserts		
354443	BD BioCoat™ Matrigel™ Matrix Cell Culture Inserts	0.4 µm inserts in four 6-well plates
354447	BD BioCoat Matrigel Matrix Cell Culture Inserts	0.4 µm inserts in two 24-well plates
EXTRACELLULAR MATRIX PRODUCTS		
BD Matrigel™ Basement Membrane Matrix		
356234	BD Matrigel Matrix	5 ml
354234	BD Matrigel Matrix	10 ml
356235	BD Matrigel Matrix (50 ml)	5x10 ml
354248	BD Matrigel Matrix High Concentration (HC)	10ml
356237	BD Matrigel Matrix phenol red-free	10 ml
354262	BD Matrigel Matrix High Concentration (HC) Phenol-Red Free	10 ml
356230	GFR BD Matrigel Matrix	5 ml
354230	GFR BD Matrigel Matrix	10 ml
354263	BD Matrigel Matrix High Concentration (HC) Growth Factor Reduced	10 ml
354277	BD Matrigel hESC-Qualified Matrix	5 mL/vial
356231	GFR BD Matrigel Matrix phenol red-free	10 ml
354235	Dispase	100 ml
354253	BD Cell Recovery Solution	100 ml
354237	Extracellular Matrix, human	1 mg

Issues with Cryopreserved Hepatocytes for Induction

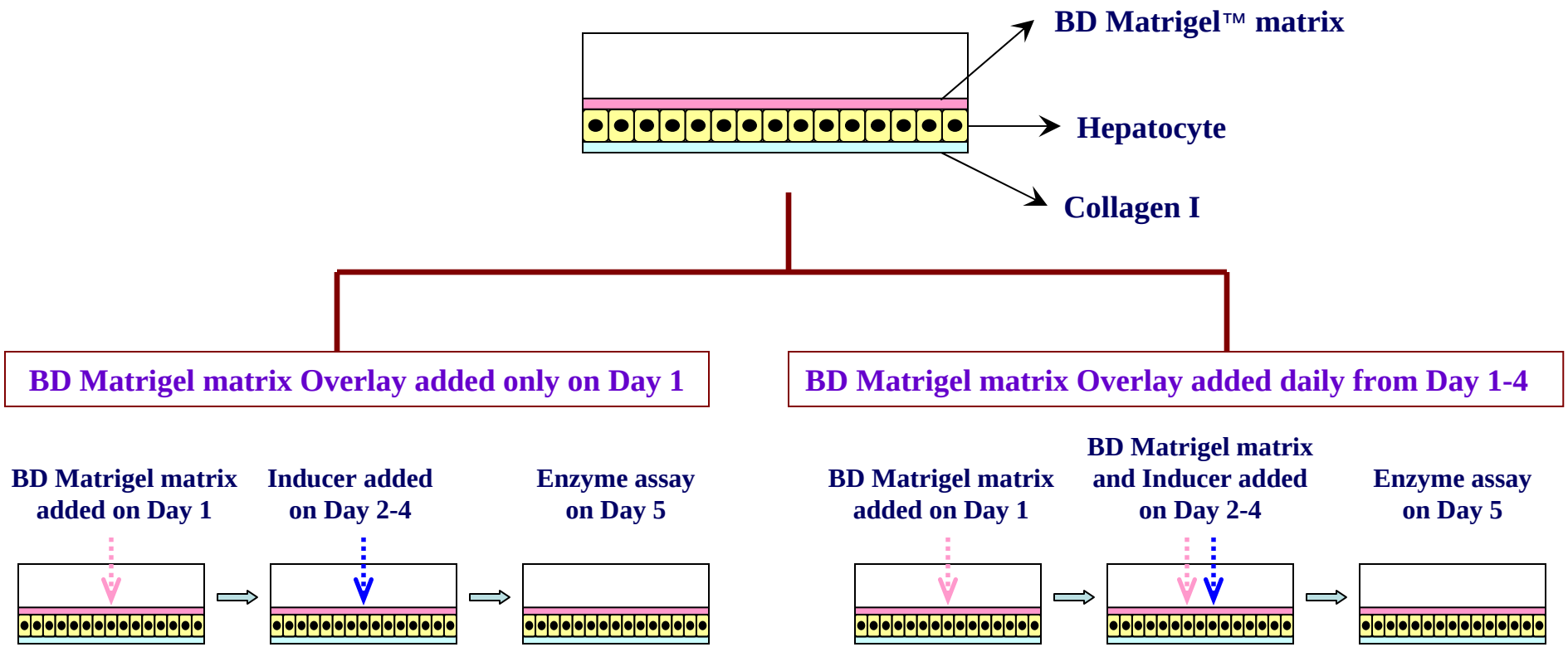
- Attached cells may not form stable monolayer
- Cells have good initial cell attachment (i.e. >70%) but low basal activity, resulting in abnormally high fold induction
  - Low basal activity can be a problem for assays with low sensitivity

Objectives

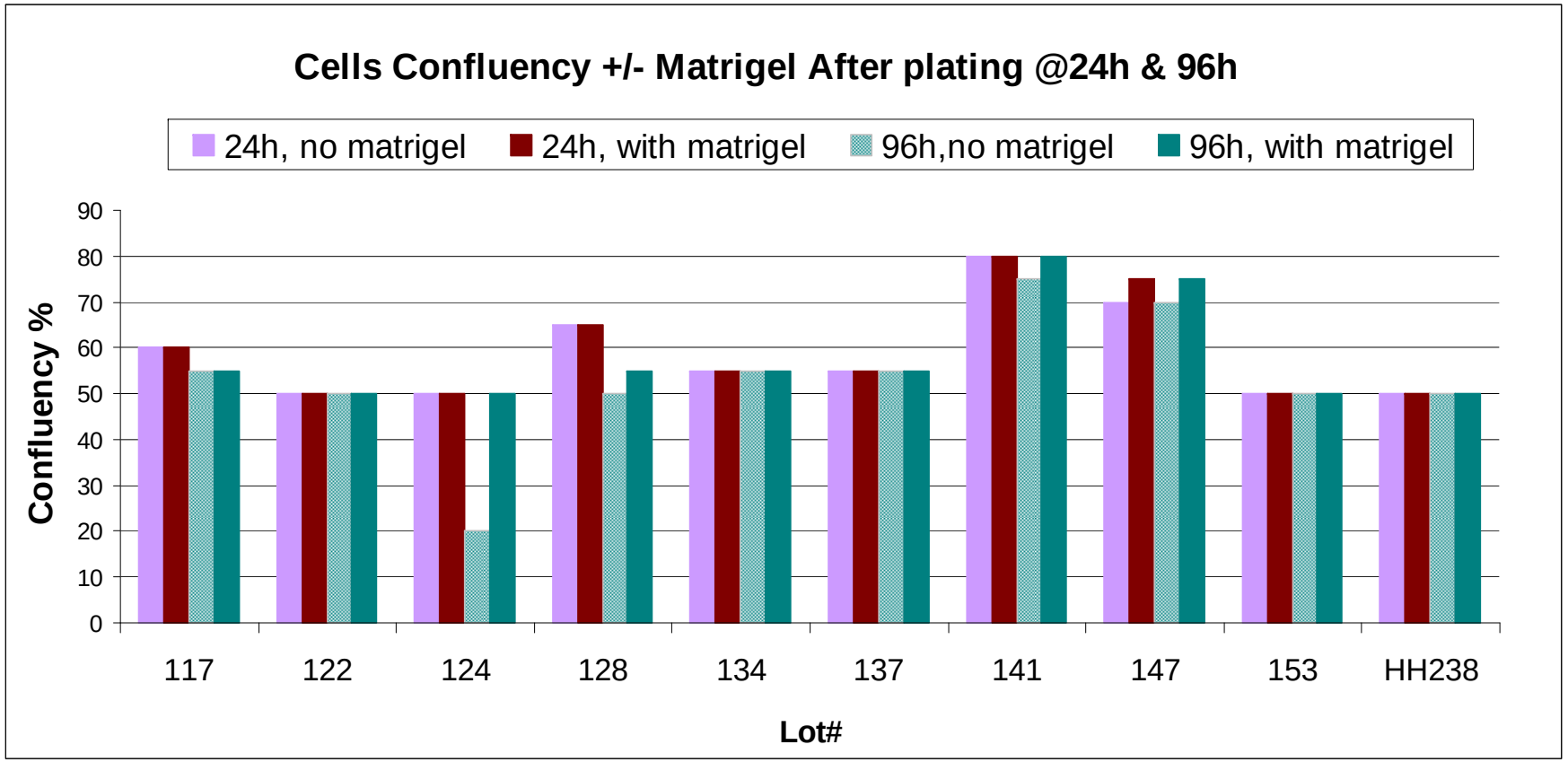
- To determine if BD Matrigel™ matrix can….
- Improve cell attachment and morphology
- Improve basal activity
- Improve induced activity
- What is the effect of BD Matrigel matrix on fold-induction?

BD Matrigel Matrix Overlay Format

Hepatocyte Collagen/BD Matrigel Matrix Sandwich Culture



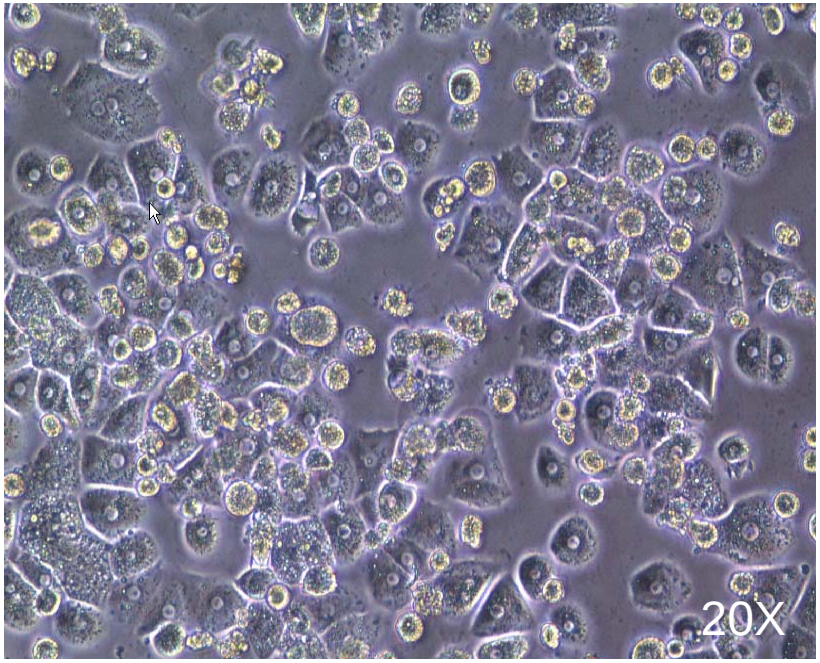
Attachment (%) at 24 and 96 Hours After Plating



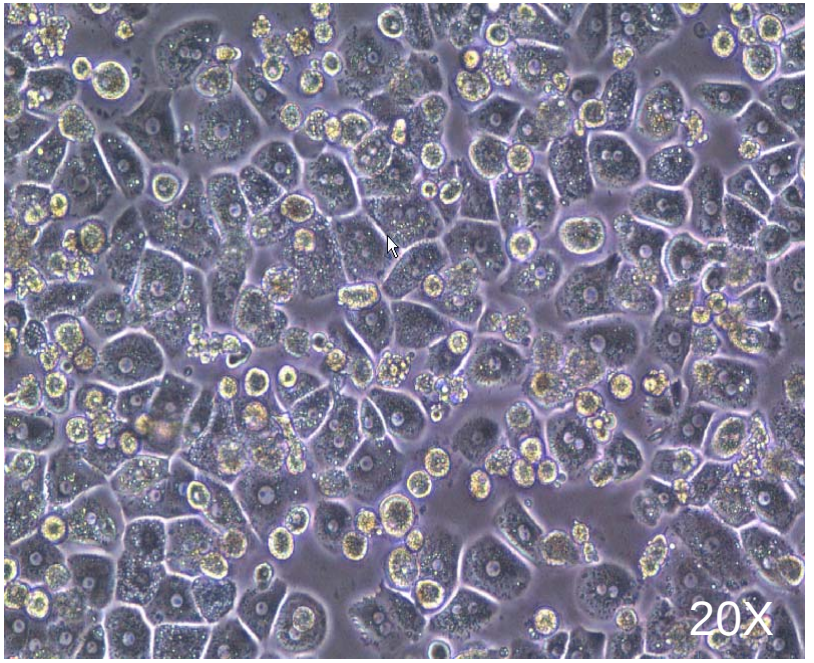
- BD Matrigel™ matrix overlaying generally did not change cell attachment

BD Matrigel Matrix Overlay Maintained Cell Morphology of Human CryoHepatocytes

Without BD Matrigel Matrix, 24 hours

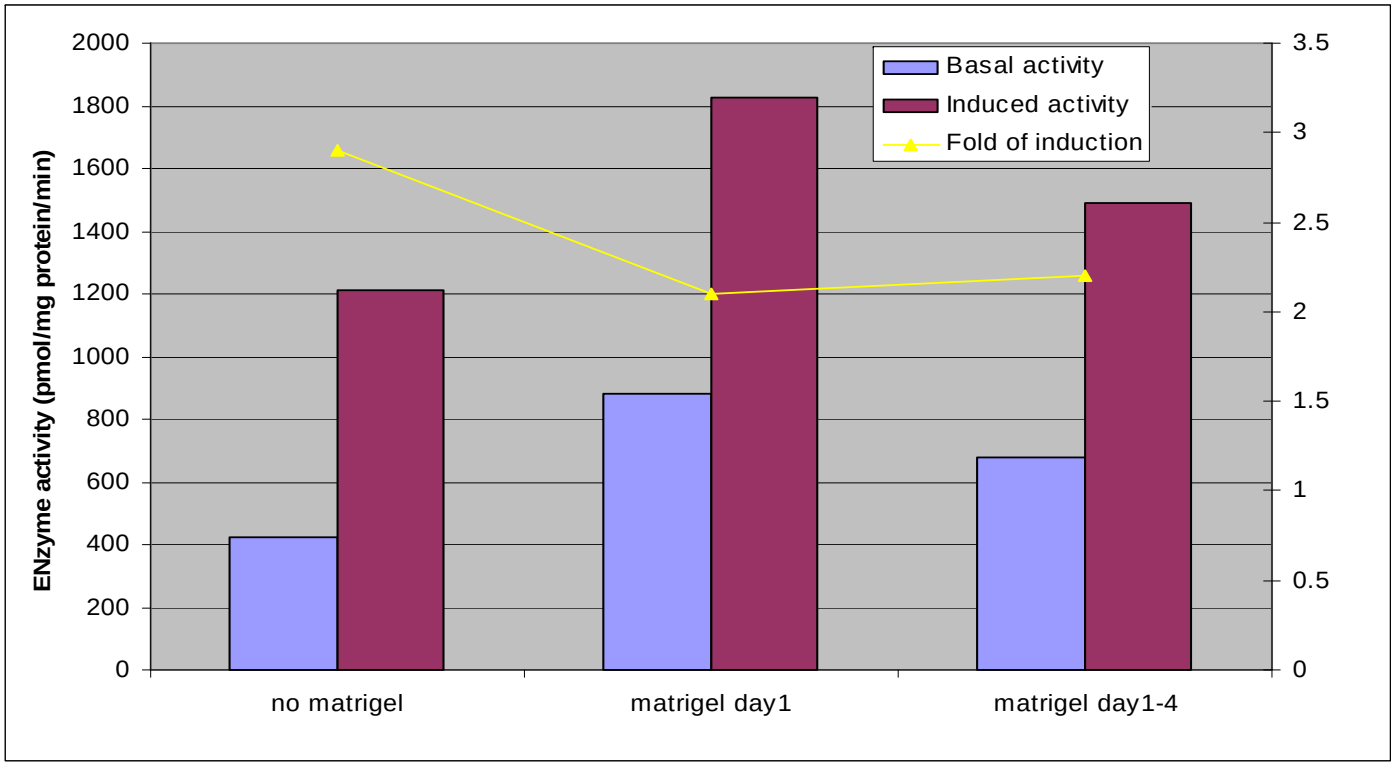


With BD Matrigel Matrix, 24 hours



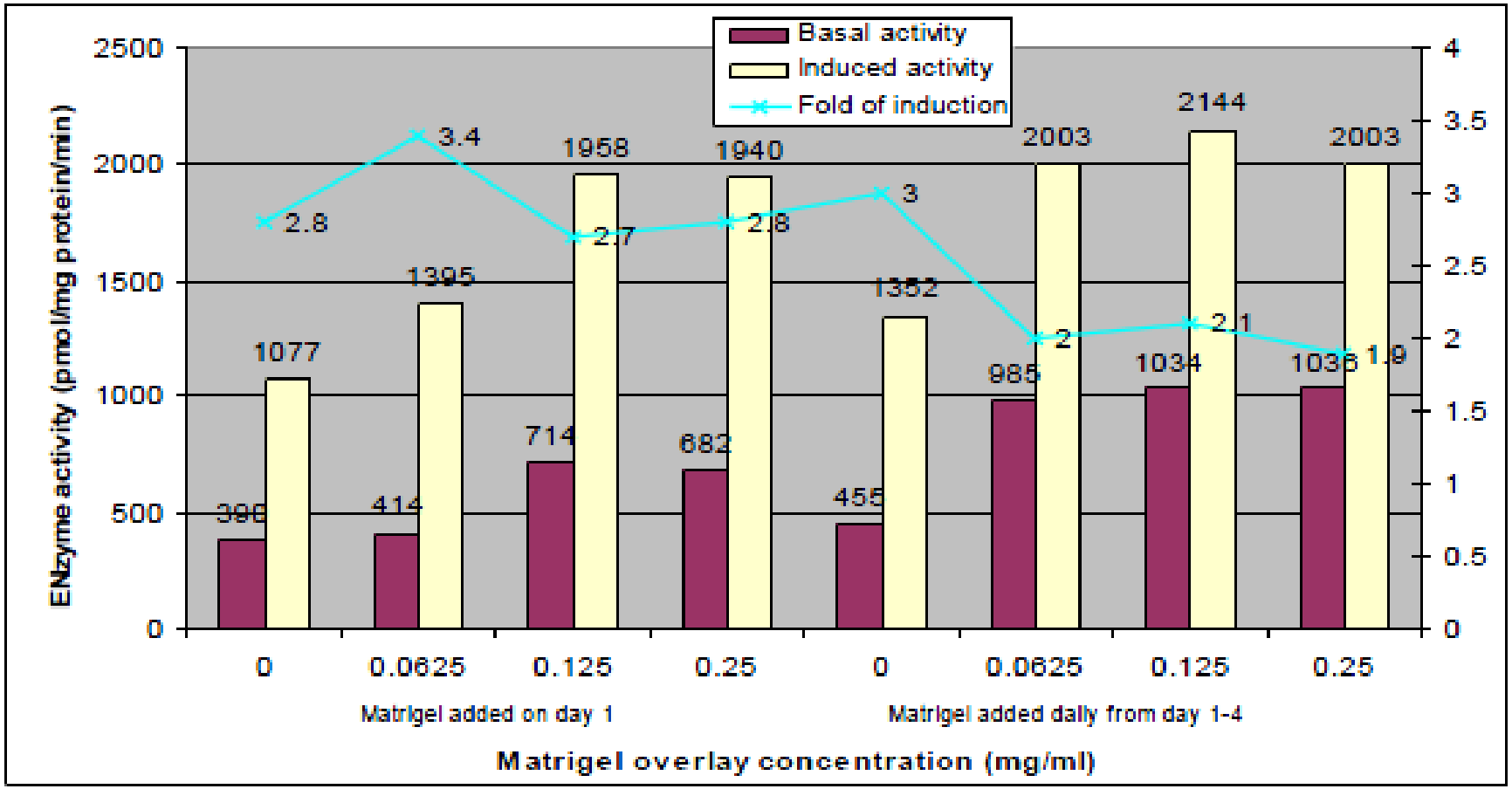
- BD Matrigel™ matrix overlaying slightly improves cell morphology

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 BD Matrigel Matrix Overlay Results

- BD Matrigel™ matrix overlay did not change cell attachment efficiency
- BD Matrigel matrix overlay improved both basal and induced CYP3A4 activities.
- Improved cell morphology slightly.

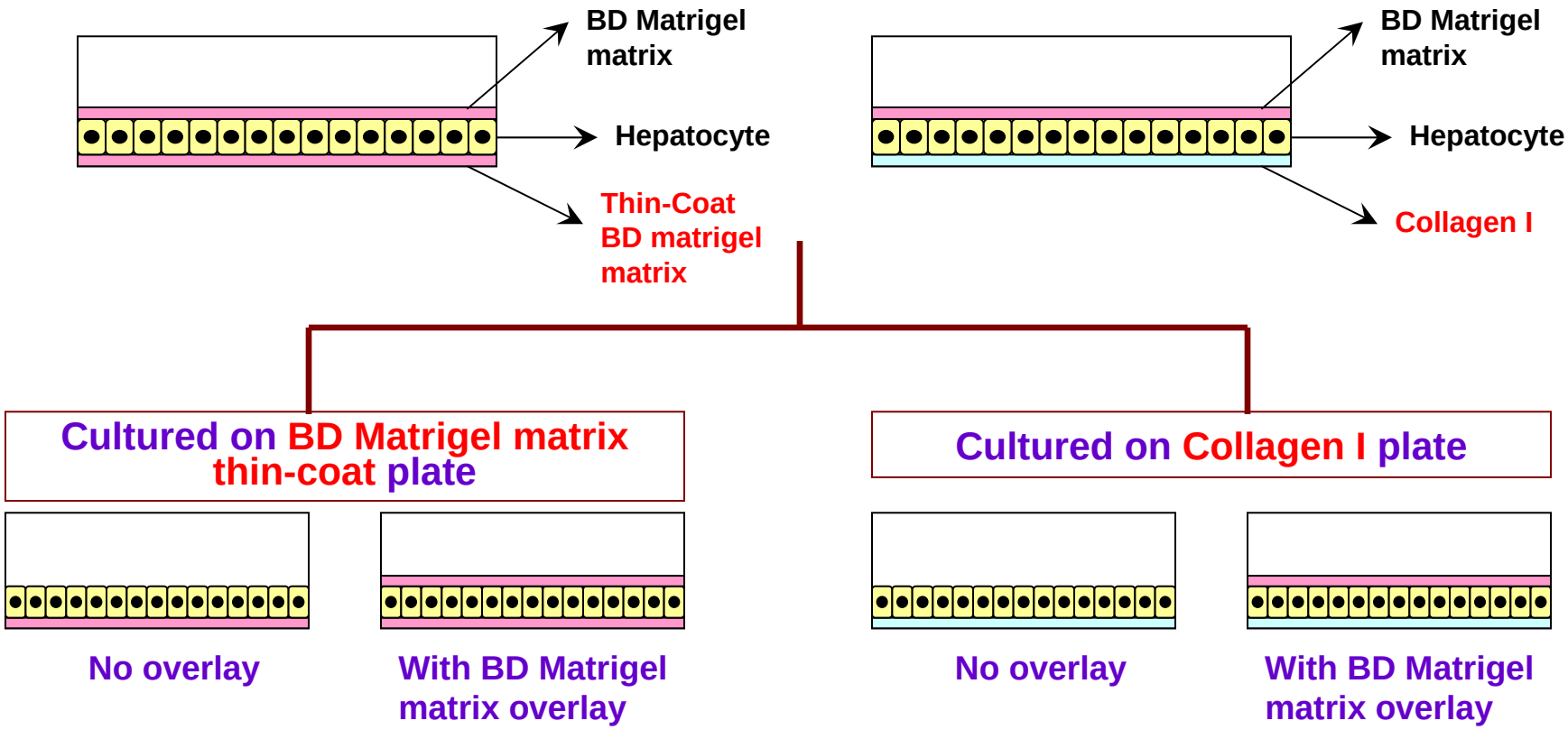
Effect of BD Matrigel™ Matrix Thin Coat on Cryopreserved Hepatocyte Function

Objective

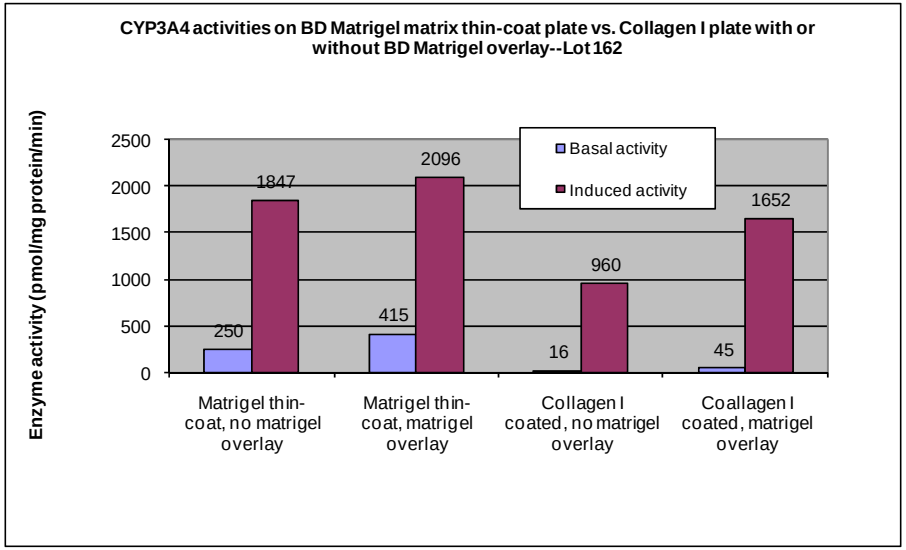
- Determine if BD Matrigel™ matrix thin-coat surface alone, or in combination with BD Matrigel matrix overlay, can improve hepatocyte basal or induced activity, fold-induction and cell morphology.

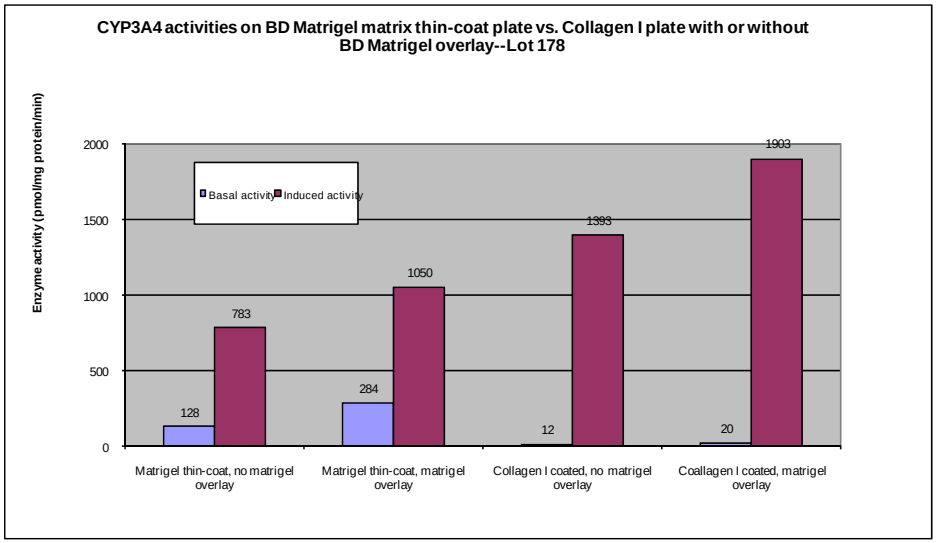
ECM Coating/Overlay Effect on CYP450 Activities – Design of Experiments

Hepatocyte Collagen/BD Matrigel™ matrix Sandwich culture



Result: BD Matrigel Matrix Coating and Overlay Effect on CYP3A4 Activities of Human CryoHepatocytes



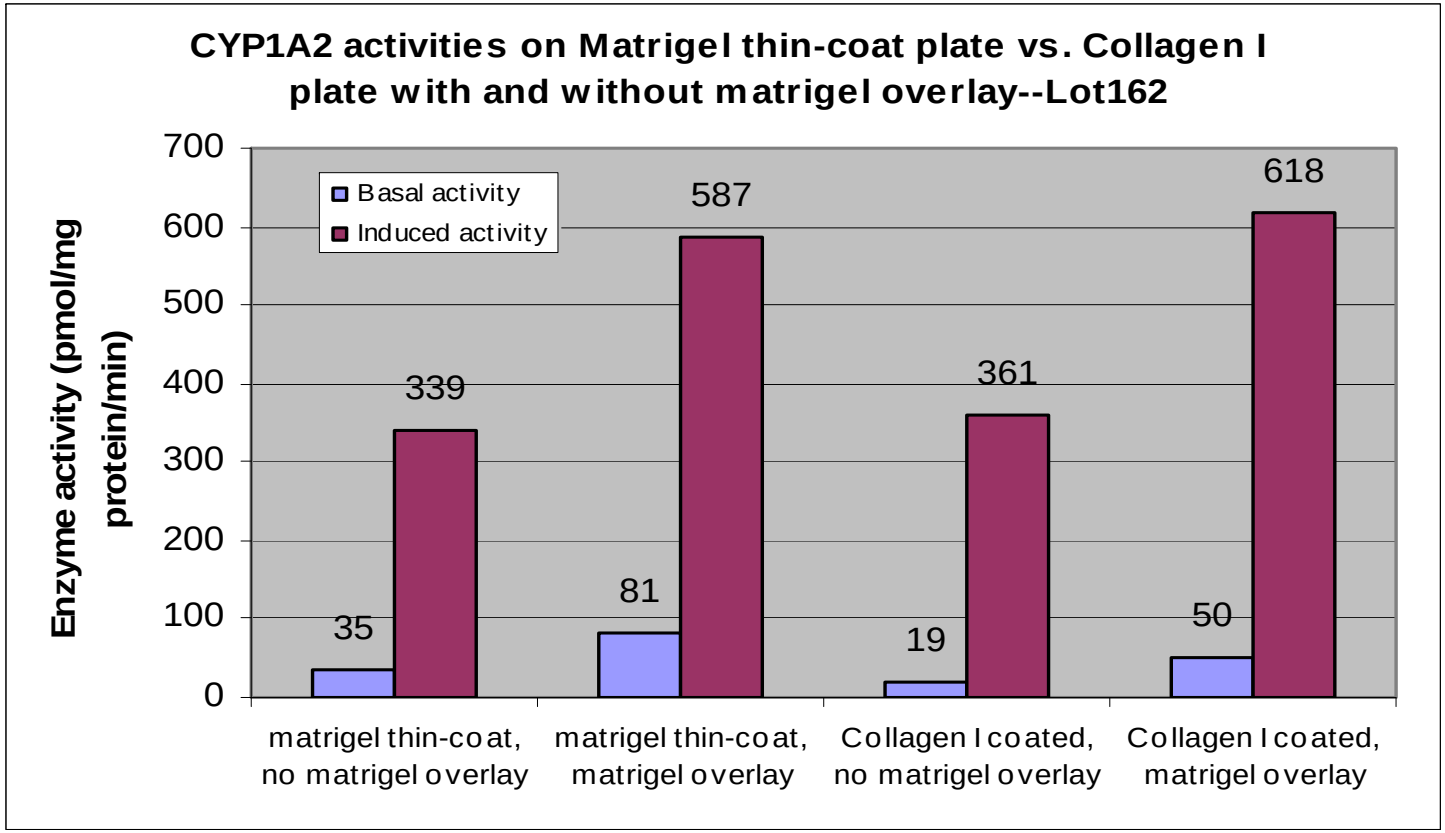


Exp. Condition	Fold-Induction
BD Matrigel matrix Thin-Coat Coated Plate	7
BD Matrigel matrix Thin-Coat/+ Matrigel Overlay	5
Collagen I Coated Plate	60
Collagen I Coat/+ BD Matrigel matrix Overlay	37

Exp. Condition	Fold-Induction
BD Matrigel matrix Thin-Coat Coated Plate	6
BD Matrigel matrix Thin-Coat/+ Matrigel Overlay	4
Collagen I Coated Plate	114
Collagen I Coat/+ BD Matrigel matrix Overlay	94

- **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.**

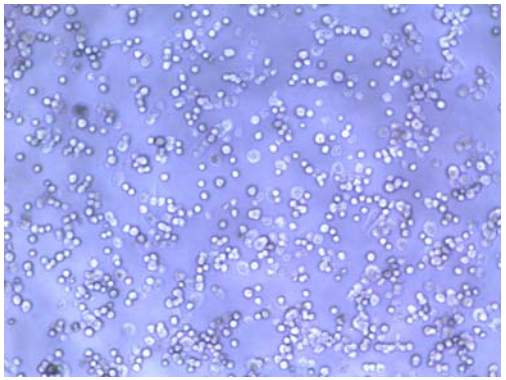
Result: BD Matrigel™ Matrix Coating and Overlay Effect on CYP1A2 Activities of Human CryoHepatocytes



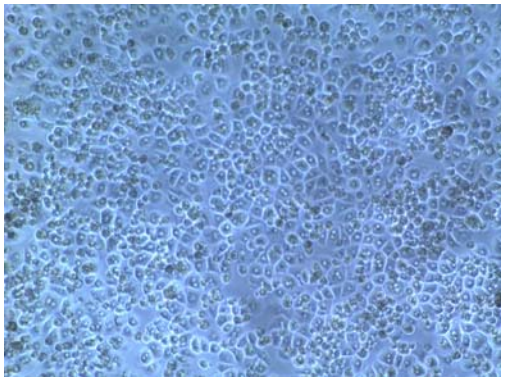
Exp. Condition	Fold-Induction
Matrigel Thin-Coat Coated Plate	10
Matrigel Thin-Coat/+ Matrigel Over	7
Collagen I Coated Plate	19
Collagen I Coat/+ Matrigel Overlay	12

Morphology Change in Day 1

7 hours

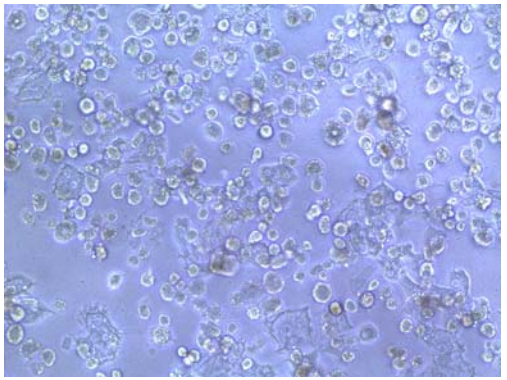


BD Matrigel matrix thin-coat
7 h, 10x, lot 178

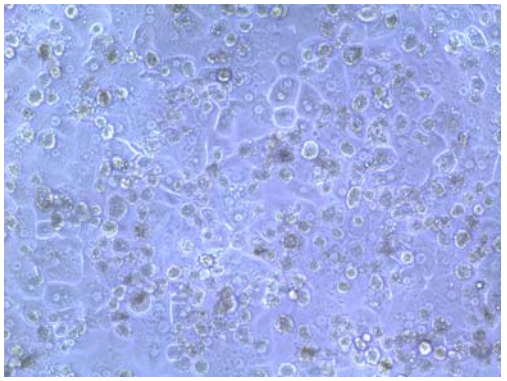


Collagen I-coated, 7 h, 10x, lot 178

24 hours



BD Matrigel matrix thin-coat
24 h, 20x, lot 178

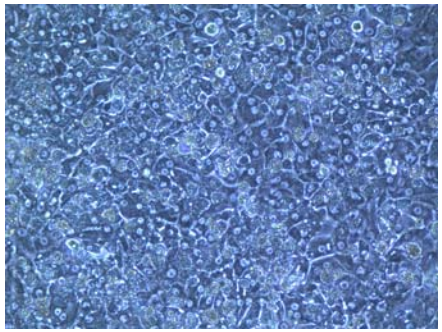


Coallgen I-coated, 24 h, 20x, lot 178

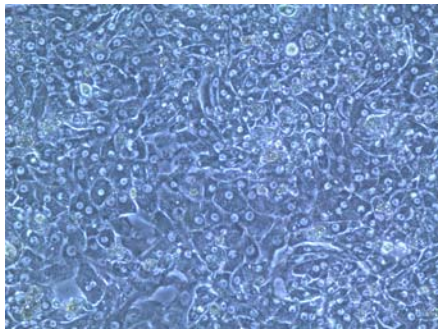
- **On collagen I surface, hepatocytes quickly spread and show typical cuboidal morphology in less than 7 hours; while on BD Matrigel™ matrix surface, hepatocytes remained spherical and formed cell aggregates for at least 24 hours**

Effect of ECM Coating and Overlay on Cell Morphology at 96 hours

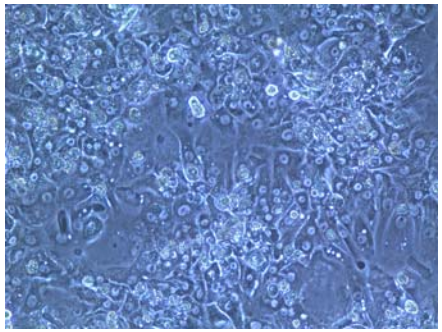
Lot 162



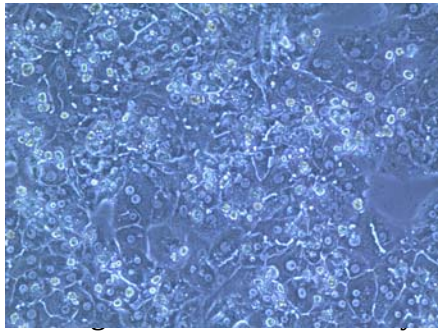
BD Matrigel matrix thin-coat, no overlay, 96 hr, lot 162, 20x



BD Matrigel matrix thin-coat, with overlay, 96 hr, lot 162, 20x

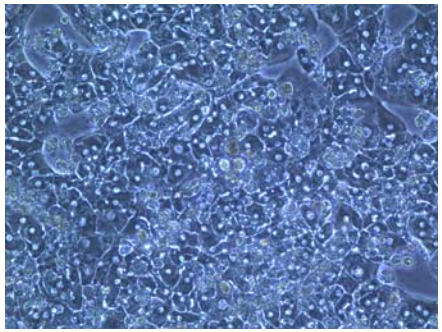


Collagen I-coated, no overlay, 96 hr, lot 162, 20x

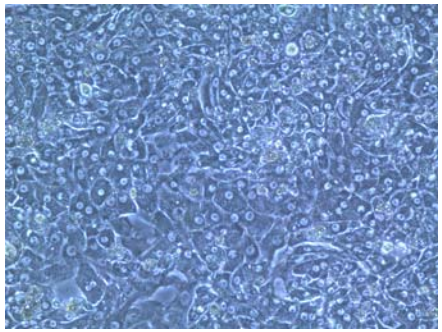


96 hr, lot 162, 20x

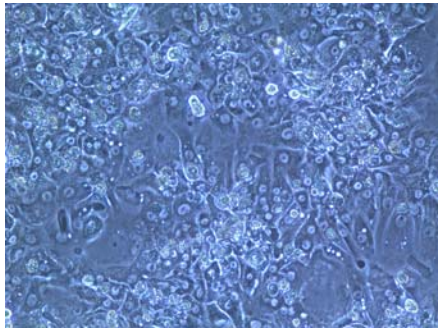
Lot 178



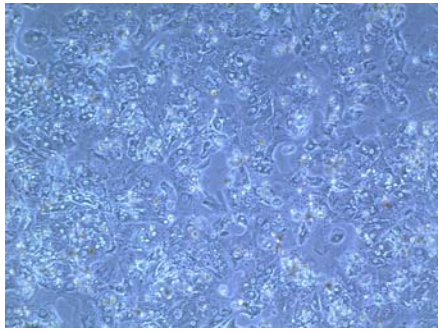
BD Matrigel matrix thin-coat, no overlay, 96 hr, lot 178, 20x



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 surface**

Conclusions

- BD Matrigel™ matrix thin-coat maintained hepatocyte morphology and 3D structure for a longer time than Collagen I 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….may facilitate applications such as chronic tox studies.

Questions?

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