Liver BioMatrix Differentiates Stem/Progenitor Cells into Mature Functional Hepatocytes

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ABSTRACT

Human fresh and cryopreserved hepatocytes plated on collagen I coated plates sometimes overlaid with Matrigel are used routinely throughout Drug Discovery for studies involving drug metabolism, transporters, gene expression profiling, induction/inhibition and toxicity screens. While being the gold standard, these cellular models are met with limitations. Here we provide data to demonstrate that human stem/progenitor cell-derived hepatocytes may offer a better model system with fewer limitations than the current gold standard models.

We have developed a hepatic model system that includes human stem/progenitor cells, novel formulations of lineage stage-specific media for expansion and differentiation of stem/progenitor cells and maintenance of mature hepatocytes as well as Liver BioMatrix isolated from decellularized liver. Our decellularization methods preserve the matrix biochemistry and structure, retaining >95% of its collagens and most of the liver's collagen-associated matrix components, growth factors and cytokines. Consequently, when human stem/progenitor cells were plated onto the biomatrix, they differentiated into mature hepatocytes within a few days without adding exogenous growth factors. Data will be presented showing that CYP3A4 activity was equivalent to primary adult hepatocytes, sustained over a period of several weeks and didn't rapidly decline as with primary adult hepatocytes. Data from other metabolic activity studies will also be presented.

MATERIALS AND METHODS

Sourcing of Human Liver Tissue – All human liver tissues were obtained with the proper informed consent. Neonatal livers were obtained from federally designated organ procurement organizations and fetal liver tissues were obtained from Advanced Biological Resources.

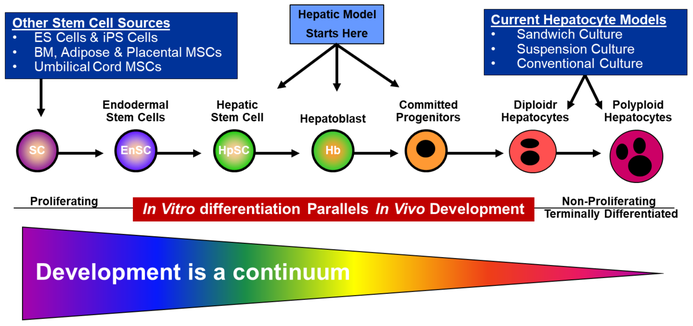
Isolation of Human Hepatic Progenitors – Human neonatal livers were perfused through the portal vein with EDTA-containing buffer for 15 min and 125 mg/L CIzyme (VitaCyte, Indianapolis, IN) for 30 min at 34°C. Cells were passed sequentially through filters of pore size 1,000, 500, 250, and 150 µm, and centrifuged in Optiprep density gradients at 500g to select for viable cells. Fetal livers were minced and digested by a digestion buffer that included a base medium of Kubota's StemCell Growth Media (PhoenixSongs) supplemented with 0.06% (w/v) collagenase, 0.03% (w/v) deoxyribonuclease, 0.5mM EDTA (all from Sigma) at 37°C. Hematopoietic cells and non-parenchymal cells were separated from the parenchymal cells by slow speed centrifugation (30g for 5 minutes in 40ml wash buffer). Cell suspension was then filtered through a 70µm nylon filter and centrifuged in Optiprep density gradients at 500g to select for viable cells.

Differentiation of Hepatic Stem/Progenitor Cells on Giga-Matrix – Hepatic progenitors were dissociated with collagenase, washed with Wash Buffer (PhoenixSongs) twice and plated onto Liver BioMatrix or onto collagen I coated plates (BD BioCoat) in Kubota's Hepatoblast Growth at a density of 150-200k cells/cm^2 Kubota's Hepatoblast medium (PhoenixSongs), followed by feeding HCM (PhoenixSongs) on day 5 post plating and then daily thereafter.

Cryopreserved Hepatocytes – Adult hepatocytes were obtained from CellzDirect and plated at densities per vendor instructions onto Liver BioMatrix or onto collagen I coated plates (BD BioCoat) in HCM (PhoenixSongs). Hepatocytes were fed HCM daily.

Characterization of the differentiated hepatocytes – CYP 3A4 activity was measured using Promega P450-Glo™ assays.

Stem Cell-Derived Hepatocytes



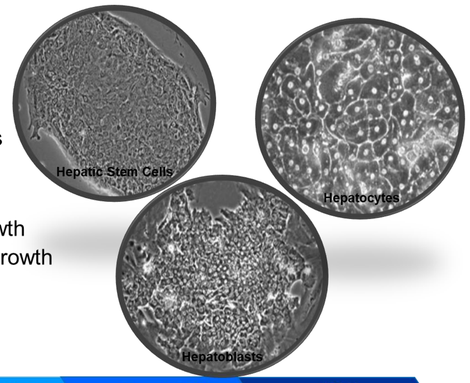
- Hepatoblasts are already committed to hepatic/biliary lineages
- Hepatoblasts on biomatrix differentiate into hepatocytes in a few days
- Cost effective – no need for exogenous growth factors to direct differentiation or weeks of differentiation time

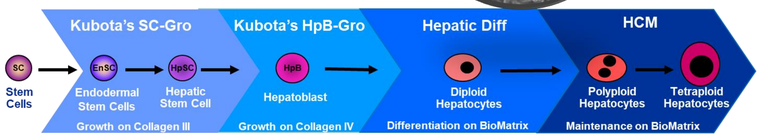
GigaCyte

Stem/Progenitor-Derived Hepatic Model

Model Includes

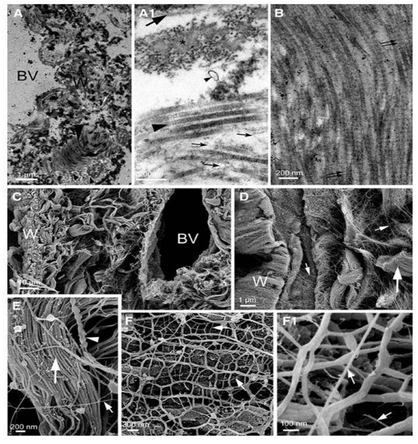
- Hepatic Cells
  - Hepatic Stem Cells
  - Biliary Tree Stem Cells
  - Hepatoblasts
- Hepatic Media
  - Kubota's StemCell Growth
  - Kubota's Hepatoblast Growth
  - Hepatic Differentiation
  - Hepatocyte Culture
- Liver BioMatrix





Preparing Liver BioMatrix

Biomatrix Scaffolds



Matrix Structure Preserved

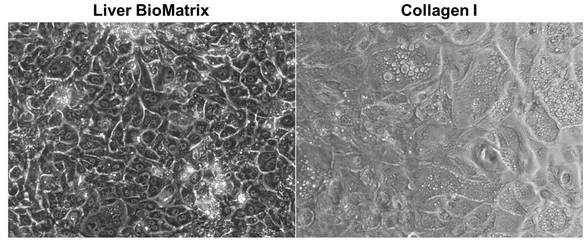
- Collagens (I,III,IV,VI,XVIII)
- Elastin
- Laminins
- Fibronectins
- Perlecan (HS-PG form)
- Entactin (Nidogen)
- Syndecans
- Glypicans
- Chondroiton sulfate PGs
- Dermatan sulfate PGs

Growth Factors are bound to the biomatrix

- FGFs, EGF, HB-EGF, GCSF, GDNF, GMCSF, MCSF,
- IFGBPs, IGF-I & II, PDGFs, TGFs, VEGFs,

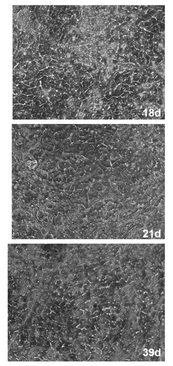
Liver BioMatrix Directs Differentiation

Hepatic Progenitors Differentiated 24 Days

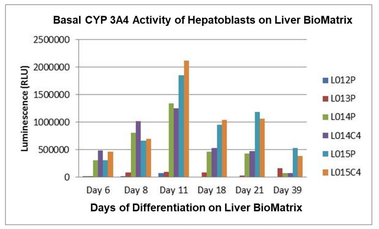


Formation of bile canaliculi human heptic progenitors differentiated into hepatocytes on Liver BioMatrix. On day 4 post plating on BioMatrix, cultures were incubated with 5M CDFDA for 20 min. CDFDA is hydrolyzed to fluorescent CDF inside the hepatocytes, which is then transported into bile canaliculi via MRP2. Red arrows show examples of bile canaliculi.

Differentiation on Liver BioMatrix



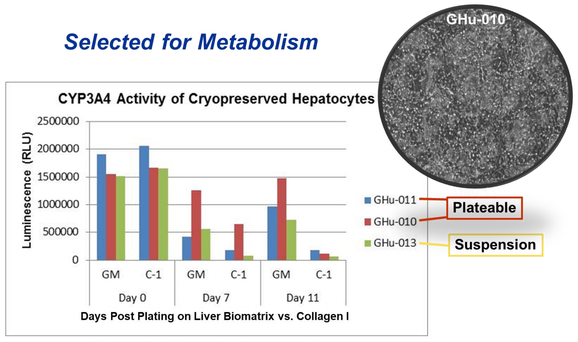
Basal Activity of Differentiated Hepatoblasts



- Hepatoblasts plated on BioMatrix in Kubota's Hepatoblast Growth media
- Fed Hepatic-Diff on day 2 post plating
- Fed HCM on day 5 post plating

Cryopreserved Hepatocytes on Liver BioMatrix

Selected for Metabolism



Enabling Drug Discovery

DMPK

- Steady state drug metabolism
- Mechanism of action

Drug-Drug Interactions

Biliary Influx, Efflux and Disposition

Long-term Tox Studies

- Longevity in culture – dose compound over time

Virology

- Hepatitis C & B infectivity & virus production

Compound Screens