Expanded Primary Hepatocytes: Achieve More Predictive Toxicity Studies

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Senior Product Line Business Manager, ATCC

Credible Leads to Incredible™
About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World’s largest, most diverse biological materials and information resource for microbes – the “gold standard”
- Innovative R&D company featuring gene editing, microbiome, NGS, advanced models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 18 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees
Agenda

- Heptocyte models
- The upcyte® solution
- upcyte® Hepatocyte characterization
- Applications
- Summary
## Problems of current hepatocyte models

<table>
<thead>
<tr>
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<th>Continuous Cell Lines</th>
<th>Stem Cell-derived Cells</th>
<th>Primary Hepatocytes</th>
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<td>Physiology</td>
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# The upcyte® Hepatocyte solution

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The upcyte® solution – a better cellular model

- Continuous cell lines
- Stem cell-derived hepatocytes
- Primary hepatocytes

Availability vs. Physiological relevance
I. Development

II. Production

III: Quality control

cell isolation  gene selection  lentiviral transduction  expansion  analysis  upcyte® cells
upcyte® Hepatocytes display adult phenotype

upcyte® hepatocytes express cytokeratin 8 (CK8), cytokeratin 18 (CK18), human serum albumin (HSA), α-anti-trypsin (AAT), but lack embryonic markers such as α-fetoprotein (AFP). The cells further expressed E-cadherin and demonstrated marked capability for glycogen storage (PAS staining) and bile secretion (CDFDA staining).
**upcyte® Hepatocytes maintain metabolic activity**

### Phase I Activity [pmol/min/mg]

<table>
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<tr>
<th></th>
<th>Donor 10-03</th>
<th>Donor 151-03</th>
<th>Donor 422A-03</th>
<th>Donor 653-03</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>3.3 ± 0.4</td>
<td>0.7 ± 1.4</td>
<td>2.3 ± 0.1</td>
<td>17.1 ± 0.5</td>
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<tr>
<td>CYP2B6</td>
<td>40.3 ± 6.5</td>
<td>71.1 ± 11.3</td>
<td>33.6 ± 11.4</td>
<td>68.4 ± 18.4</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>91.8 ± 5.5</td>
<td>29.1 ± 21.4</td>
<td>4.8 ± 3.1</td>
<td>16.2 ± 0.9</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>21.4 ± 9.6</td>
<td>77.8 ± 22.6</td>
<td>42.9 ± 6.3</td>
<td>178.3 ± 17.0</td>
</tr>
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### Phase II activity [pmol/min/mg]

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<td>SULT (Hydroxycoumarin)</td>
<td>6-16</td>
<td>5-98</td>
</tr>
<tr>
<td>UGT (Hydroxycoumarin)</td>
<td>32-345</td>
<td>15-496</td>
</tr>
<tr>
<td>GST (CDNB)</td>
<td>15-88</td>
<td>21-35</td>
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Data supplied by upcyte® technologies.
**upcyte® Hepatocytes – metabolism**

**Induction** of CYP3A4 (by rifampicin)
Donors 10-03 and 422A-03

**Inhibition** of CYP3A4 (by ketoconazole)
Donor 653-03

Data supplied by upcyte® technologies.
Comparison of the TC$_{50}$ of 18 model compounds in upcyte® Hepatocytes and primary human hepatocytes. Toxicity was measured using the MTS assay. All donors showed an R$^2$ correlation of 0.99 (n=3).

Cytotoxicity – acute and repeated-dose studies

Employed compounds:
- CIT: Sodium citrate (1-2 mM)
- APAP: Acetaminophen (0.5-2 mM)
- TROG: Troglitazone (50-100 µM)
- CYC(A): Cyclosporin A (20-50 µM)
- CHP: Cumene hydroperoxide (100-500 µM)
- ROT: Rotenone (0.05-1 µM)

upcyte® Hepatocytes were exposed to test compounds for 24 h or 1 week. (A) viability, (B) apoptosis, (C) changes in mitochondrial membrane potential (MMP), (D) production of mitochondrial superoxide, (E) ROS (F) intracellular Ca$^{2+}$ levels using HCS.

Clearance prediction

The reference drug set:
- Alprazolam (1)
- Prednisolone (2)
- Diazepam (3)
- Voriconazole (4)
- Tolbutamide (5)
- Meloxicam (6)
- Warfarin (7)
- Glimepiride (8)
- Riluzole (10)
- Oxazepam (11)

Good correlation between predicted $\text{CL}_H$ and observed in vivo CL values was observed for the subset of low CL drugs (shown here). $\text{CL}_H$ for 73% (8 of 11 compounds) were predicted within twofold of in vivo $\text{CL}_{\text{nonrenal}}$. 

Transfection – transfection with a GFP construct

Transfection mediated using nucleofection.

GFP transfected, < 67% efficiency achieved.

Data supplied by upcyte® technologies.
Transfection – lipid delivery system

**Primary hepatocytes:** < 5% positive cells

**upcyte® Hepatocytes:**
Up to approximately 50% transfection efficiency possible as demonstrated by GFP expression and flow cytometry.

Data supplied by upcyte® technologies.
upcyte® Hepatocytes – long term cultures

Long term culture offers new possibilities

Data supplied by upcyte® technologies.
Product format

**upcyte® Hepatocytes (ATCC® ACS-9000™)**
5 x10⁶ cells per vial

Cells tested for:
- Cell morphology
- > 70% viable recovery
- > 90% plating efficiency
- Markers: CK8+, CK18+; HSA+, AAT+ (α-1-antitrypsin), AFP- (α-fetoprotein)
- Capacity for glycogen storage (PAS staining)
- Basal and inducible CYP activities (Phase I)
Product format

**upcyte® Hepatocyte Performance Media Kit (ATCC® ACS-9005™)**

**A multi-component kit:**

- Hepatocyte Performance Medium (ATCC® ACS-9001™; 500 ml) stored at 2-8°C
- Hepatocyte Performance Medium Supplements, (ATCC® ACS-9002™) stored at -20°C
  - Supplement A (proprietary formulation, ATCC® ACS-9003™; 5ml)
  - L-glutamine, (ATCC® ACS-9004™; 5ml)

- Kit components are tested for sterility, mycoplasma, and pH
- Complete medium is tested for growth performance
  - Typical morphology
  - Adherence
  - Growth rate
- Once supplemented, complete medium is stable for 1 month at 2-8°C
Summary – the upcyte® solution

Primary cell features
- Generated from healthy human adult cells
- Karyotypically stable
- Physiologically relevant profile
- Cell type-specific phenotype

Extensive availability
- Up to 3000 vials from a single donor
- Supply for screening applications

Flexible use
- 2D & 3D
- Co-culture with other cell types
- Long-term cultures
Summary – the upcyte® solution

**Easy & safe handling**
- Quality controlled cells
- Detailed information on cell type specifications
- Standardized procedures for use
- Optimized media

**Wide range of applications**
- Basic R&D
- Pharmaceutical preclinical development
- ADMET, viral infections
Thank you for your attention. Questions?
Cultivating collaboration to support global health

Visit www.atcc.org/expandedhepatocytes for more information

Visit us at SOT 59th Annual Meeting & TOXEXPO,
- March 15-19, 2020, Anaheim, CA
- Booth #463
- Exhibitor-hosted Session: Immortalized Hepatocytes from ATCC with Full Functionality and Unlimited Availability

www.atcc.org/webinars