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Primary Liver Endothelial Cells

Importance of Passage Number in Measuring the Uptake of Large Molecules

Maureen Bunger, Katherine Dunnick;
Lonza Walkersville, Inc., Walkersville, MD, USA

Introduction

Liver endothelial cells (LEC) act as the primary barrier lining the blood vessels and sinusoids of the liver. A unique subtype of LECs, liver sinusoidal endothelial cells, are defined as a subset of endothelial cells found at the interface between the liver sinusoids and hepatocytes1 where oxygen rich blood of the hepatic artery mixes with the nutrient rich blood of the portal vein. LSECs provide a highly fenestrated permeable barrier and are distinguished from general LECs (including vascular and lymphatic endothelial cells) by the ability to absorb a broader range of molecules coming from the enteroheptic blood circulation.

Liver sinusoidal endothelial cells are a major site of antibody recycling[2,5], to the degree that much of the pharmacokinetics of antibody therapeutics can be attributed to the clearance capabilities in the liver endothelium[3]. As such, LSECs are desired for cell culture applications to assess uptake and turnover of antibody therapeutics in development. Primary LSECs can be isolated from intact livers using a combination of tissue digestion, centrifugation, and affinity purification. However, little is known about the ability of isolated LSECs cultured *in vitro* to recapitulate *in vivo* function, especially with respect to culture conditions over time.

Acetylated low density lipoprotein (ALDL) is a ~500kDa molecule naturally taken up by LSECs at faster rates than by LECs through scavenger receptor mediated uptake[4]. These scavenger receptors are a type of endocytic receptor, similar to Fc receptors important in antibody recycling[5]. Recent research has demonstrated that in cell culture, primary isolations of LSECs quickly lose their permeability to large molecules and become phenotypically similar to LECs1. In this Tech Note, we demonstrate the importance of using the early passages of isolated mixed LECs for maintaining optimal permeability function of LEC/LSECs isolated from whole human livers. Through these data, we feel that early passage mixed LECs can therefore function as a physiologically relevant *in vitro* model for large molecule uptake and recycling by the sinusoidal endothelium.

Methods

Primary LEC cells were isolated from whole liver, plated into flasks, and cryopreserved when the monolayer reached approximately 80-90% confluence. This was called passage 1.

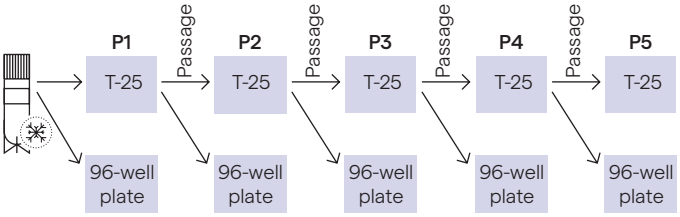

Figure 1.
Schematic method of serial passaging to determine effect of passage on ability to uptake ALDL.

Passaging cells

1. Cryopreserved LEC cells at passage 1 (P1) were thawed and plated in flasks at 10,000 cell/cm^2 using complete EGM™ 2 Medium.
2. Once the monolayer reached ~70% confluence, cells were passaged using standard trypsin methods.
3. Cells were passaged repeatedly until reaching passage 5 (P5).

Cell analysis

1. During initial thaw and after each passage of flasks, a fraction of the cells were plated in 96-well tissue culture plates at 10,000 cells/cm^2.
2. Once the monolayer reached ~70% confluence, plates were incubated with 15 µg/mL ALDL (ThermoFisher, L23380) for 3 hours at 37°C.
3. Cells were washed 3 times with PBS to remove excess ALDL, incubated with Hoechst nuclear stain, and imaged with the GFP (ex450-490/em500-550nm) and DAP (ex325-375/ em435-485nm) filter cubes.
4. This process was continued until cells reached P5 (Figure 1).

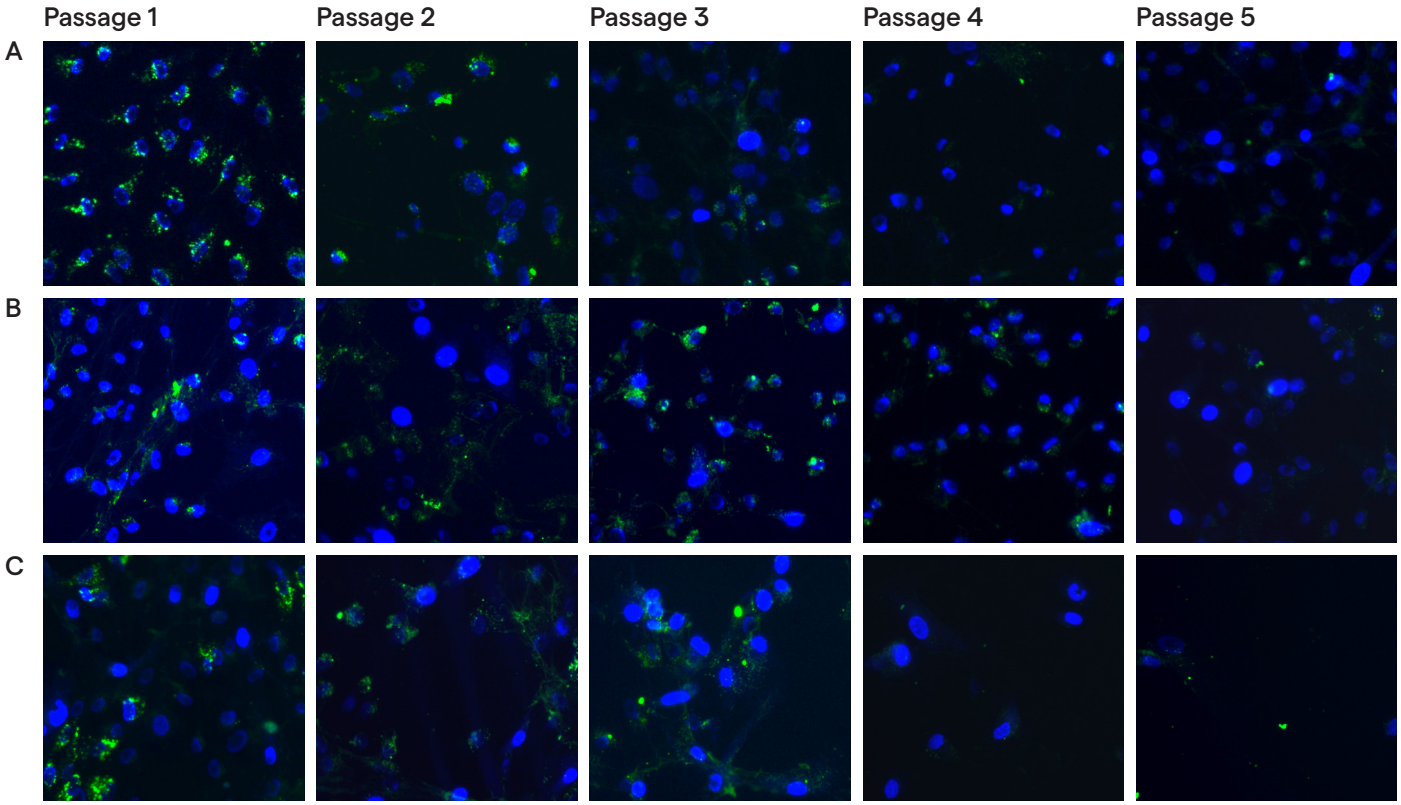

Figure 2.
LEC grown from P1 to P5 and assessed for ALDL uptake. (A, B, C) represent three separate lots of LECs. (A) 14 year old Caucasian male (B) 28 year old Caucasian male (C) 51 year old Caucasian female. Nuclei were stained with Hoechst nuclear stain (blue), while ALDL is represented by green fluorescence.

Results

LEC uptake of ALDL decreases throughout passaging

To determine the permeability of the LEC/LSEC population for large molecules, we assessed fluorescently labeled ALDL uptake over 3 hours as a function of passage number. Results demonstrate that as cells are continuously cultured and serially passaged, their ability to take-up ALDL decreases (Figure 2). Donor-to-donor variability was assessed by assaying LEC/LSECs from three independently isolated donors. Data was collected for 3 donors and demonstrates donor-to-donor variability in the rate of ALDL uptake from P1 to P5. All donors demonstrated decreased ALDL uptake by P3 and little to none by P4 (Figures 2 and 3, Table 1). Donor C showed slowed growth, preventing cells from reaching confluence by passage 5. Additionally, Donor C had declined ALDL uptake in P1 relative to Donors A and B (Table 1).

	P1	P2	P3	P4	P5
A	97	77	24	8	4
B	66	52	70	32	5
C	38	53	58	14	33

Table 1.
Relative % of cells associated with ALDL. Nuclei were counted and compared to number of nuclei associated with fluorescent ALDL. Values are percentages. Note: Donor C's slowed growth limited number of cells for ALDL uptake assessment.

ALDL uptake in LEC cells

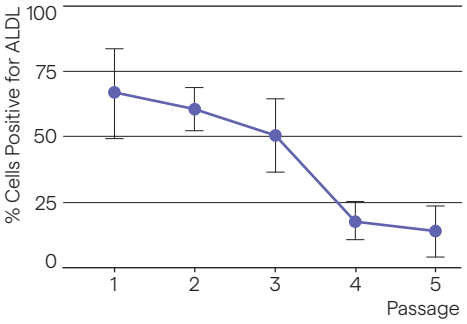

Figure 3.
Results were calculated as percentage nuclei associated with ALDL and are shown as mean ± SEM. Note: Donor C was unable to reach 70% confluence due to slowed growth in culture between passage 4 and 5.

Catalog No.	Offering
HLECP1	Human Liver-Derived Endothelial Cells P1
HLECP2	Human Liver-Derived Endothelial Cells P2
CC-3162	EGM® 2 Endothelial Cell Growth Medium-2 BulletKit®
CC-3156	EBM® 2 Endothelial Cell Basal Medium-2
CC-4176	EGM® 2 Endothelial Cell Growth Medium-2 SingleQuots® Supplements and Growth Factors

Table 2
Lonza's current liver endothelial cell and media offerings.

Liver Endothelial Cell specifications (HLECP1):

- Viability post thaw ≥70%
- Yield ≥ 1×10^6 cells/vial
- Purity ≥80%
- ALDL uptake and LSEC percentage provided as FIO

Summary

In the process of isolating and purifying endothelial cells from liver, pure populations of LSECs are difficult to obtain and maintain once in culture[2]. Therefore, providing P1 cryopreserved cells with mixed population of LEC and LSECs helps ensure the populations maintain permeability to large molecules as they would *in vivo*.

Our data indicates that P1 mixed LEC/LSEC cells take-up ALDL, indicating the presence of functional LSECs in the mixed population. Though variable between donors, continuing passage of the cells dramatically decreases their uptake and growth potential. By using mixed population of LECs at an early passage, researchers are able to recapitulate the *in vivo* function of uptake and recycling of large molecule nutrients and therapeutics in an *in vitro* setting.

Further, while ALDL can be taken up by other cells in the liver (kupffer, dendritic, etc)[2], all Human LEC Passage 1 (HLECP1) cells are screened for purity via FACS to ensure that ALDL uptake is not due to the presence of other cell types. Providing a relative uptake percentage of ALDL allows researchers to select lots with appropriate permeability and uptake capabilities for their research.

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Contact Us

North America
Customer Service: +1 800 638 8174 (toll free)
order.us@lonza.com
Scientific Support: +1 800 521 0390 (toll free)
scientific.support@lonza.com

Europe
Customer Service: +32 87 321 611
order.europe@lonza.com
Scientific Support: +32 87 321 611
scientific.support.eu@lonza.com

International
Contact your local Lonza Distributor
Customer Service: +1 301 898 7025
Fax: +1 301 845 8291
scientific.support@lonza.com

Lonza Walkersville, Inc. – Walkersville, MD 21793

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bioscience.lonza.com
www.lonza.com/lecs