Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary materials

Mimicking Liver Sinusoidal Structures and Functions using a 3D-configured Microfluidic Chip

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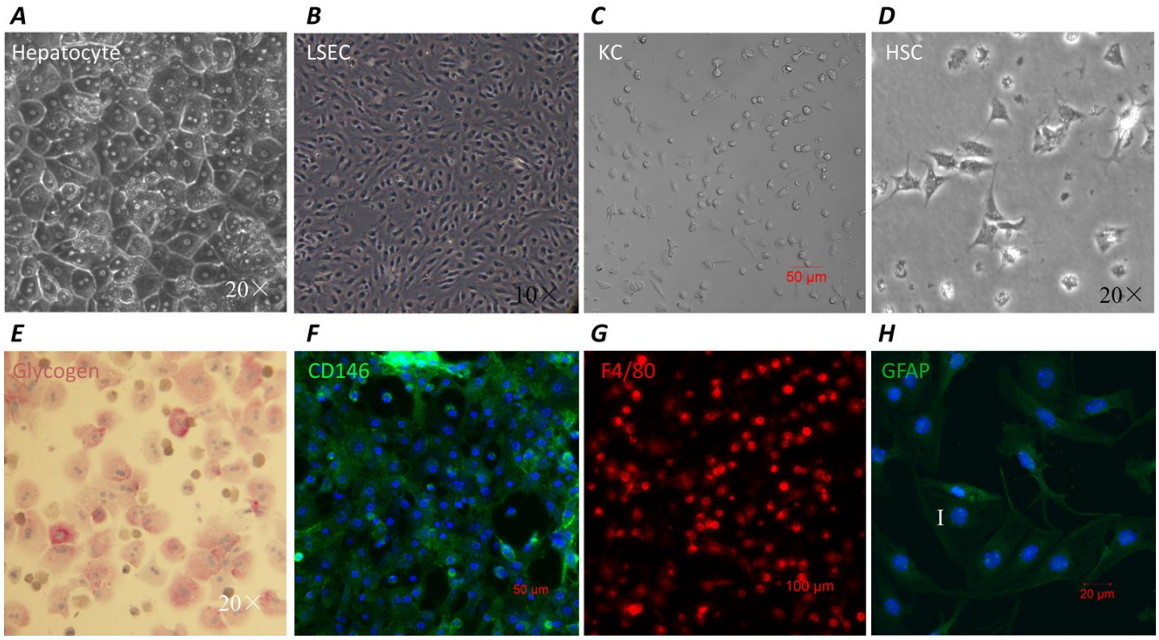

Figure S1. Characterization of four types of primary hepatic cells from mouse liver. Hepatocytes display a polygonal shape with double nucleus and clear edges (*A*) and almost all hepatocytes contain glycogen as stained in *purple* (*E*). LSECs form a monolayer presenting a cobblestone shape (*B*) and are identified with CD146 as stained in *green* (*F*). KCs yield a polymorphic shape (*C*) and are identified with F4/80 as stained in *red* (*G*). HSCs have a stellate-like shape (*D*) and are identified with GFAP as stained in *green* (*H*).

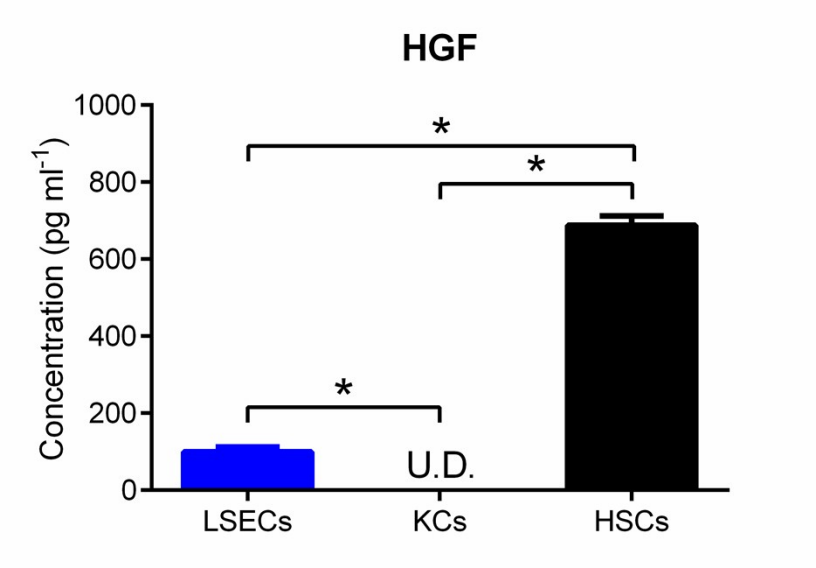

Figure S2. HGF secretion from each type of NPCs. HGF production (n = 4) were determined from the supernatant collected at 24 h after each type of NPCs are soundly attached to substrate in 96-well plate. Plotted are the mean ± SD and statistical analysis was conducted by t-test. *: $P < 0.05$.

Materials and Methods

Immunofluorescence staining and confocal microscopy

Cells were fixed with 4% formaldehyde at room temperature for 15 min after 24 h co-culture *via* dissembling the chip. Blocked and incubated with respective primary mAbs at 4°C overnight in PBS containing 1% BSA, the cells were incubated for 2 h at 37°C with secondary antibodies in the same buffer. Specific immunostaining was performed with FITC-conjugated anti-CD146 mAbs for LSECs, PE-conjugated anti-F4/80 mAbs for KCs, rabbit polyclonal anti-GFAP antibodies (Abcam, UK) for HSCs, and Alex Fluor 647-conjugated anti-CK-pan mAbs (Biolegend, CA) and rabbit anti-E-cadherin mAbs (Cell Signaling Technology, MA) for HCs, followed by FITC-conjugated donkey anti-rabbit secondary antibodies (Abcam, UK) for those stained with non-conjugated primary mAbs. In some cases, HSCs residing in the chip were stained with a highly bright dye of Cell Tracker Green (Thermo Fisher, MA) to enhance the test sensitivity. Immunofluorescence images of stained cells were examined using confocal laser-scanning microscopy (Zeiss LSM710, Germany).

ELISA assay

Culture medium was exchanged after all the four types of cells were attached well and spread completely onto the respective substrates at ~6 h. Collected supernatants after 24 h co-culture from the chip or from 96-well plate were centrifuged at 500×g, 4°C for 8 min to remove cell debris, and then stored at -80°C. ALB, hepatic growth (HGF), and

vascular endothelial growth factor (VEGF) concentrations were tested by ELISA assay kits for mouse ALB (Bethyl Laboratory, TX), HGF (R&D, MN), and VEGF (R&D, MN) in the respective sensitivity of < 1.23 ng ml^{-1}, 1.33 pg ml^{-1} and 3 pg ml^{-1}. Urea concentration was quantified by commercial urea assay kit (Stanbio Laboratory, TX).

Cytochrome P450 (CYP) activity assay

For determining CYP metabolism activities, distinct cells were cultured inside the chip in culture medium supplemented with 200 μM phenacetin (for 1A2 metabolic test) or 30 μM dextromethorphan (for 2D6 test). Those supernatants were collected after 24 h co-culture and the metabolic products of phenacetin and dextromethorphan, *that is*, acetaminophen and dextrorphan, were respectively determined by LC-MS/MS (Agilent 1200 HPLC, CA and API 4000 mass-spectrometer, CA). Substrates and metabolized products used for calibrating curves were commercially purchased from RILD Company (Shanghai).

Scanning electron microscopy

LSECs were seeded in the liver chip or a conventional dish at a density of 1.3×10^3 mm^{-2} and cultured for 1 day. Cells were fixed with 2.5% (v/v) glutaraldehyde in PBS at 4°C overnight. The samples were then washed thrice with PBS for 10 min and dehydrated in ascending ethanol concentrations (30, 50, 70, 90 and 100%) for 10 min each. Subsequently, the samples were critical-point dried using liquid CO_2 and sputter coated

with gold to avoid surface charging. Finally the specimens were investigated using a field emission (FE) SEM KYKY-EM8000F (KYKY Technology Co., Ltd, Beijing, China).

Statistical analysis

P values were calculated using the two-tailed *t*-test for two groups with equal variance if passing the normality test (Shapiro-Will) or using Mann-Whitney test if not. For multiple group comparisons, one-way ANOVA test was performed if passing the normality test or using ANOVA on RANKs if not. Statistical significance was set at $P < 0.05$.