Supplemental Data for "Transport-mediated angiogenesis in 3D epithelial co-culture" by Sudo *et al.*, *The FASEB Journal*

SUPPLEMENTAL METHODS

Analytical solution of interstitial flow velocity across a gel scaffold

Permeability of a collagen gel scaffold was measured by monitoring displacement of medium level in reservoirs. The Darcy permeability (K) of the gel scaffold with hepatocytes was then determined by Darcy's law:

$$\frac{Q(t)}{A} = \frac{K}{\mu}\frac{\Delta P(t)}{L} \tag{1}$$

where Q is the volumetric flow rate measured by the displacement of medium level in reservoirs, A is the average surface area of the gel scaffold, μ is viscosity, P is pressure, and L is the length of the gel scaffold.

The pressure in the reservoir changes with decreasing reservoir column height (Δz):

$$\Delta P(t) = \rho g \Delta z(t) \tag{2}$$

$$\Delta z(t) = \Delta z_0 - \frac{1}{A_r}\int Q(t)dt \tag{3}$$

where Δz_0 is 5 mm, ρ is density, g is gravity acceleration, and A_r is the surface area of the reservoir.

Equations (1)–(3) give

$$Q(t) = \frac{\rho g A K \Delta z_0}{\mu L} e^{-\frac{\rho g A K}{\mu A_r L}t} \tag{4}$$

$$V(t) = \int Q(t)dt = A_r \Delta z_0 \left(1 - e^{-\frac{\rho g A K}{\mu A_r L} t} \right) \tag{5}$$

where *V(t)* is displaced volume in the reservoir.

By transforming equation (5), the Darcy permeability (*K*) was given

$$K = -\frac{\mu L A_r}{\rho g A t_1} \log\left\{ 1 - \frac{V(t)}{A \Delta z_0} \right\} \tag{7}$$

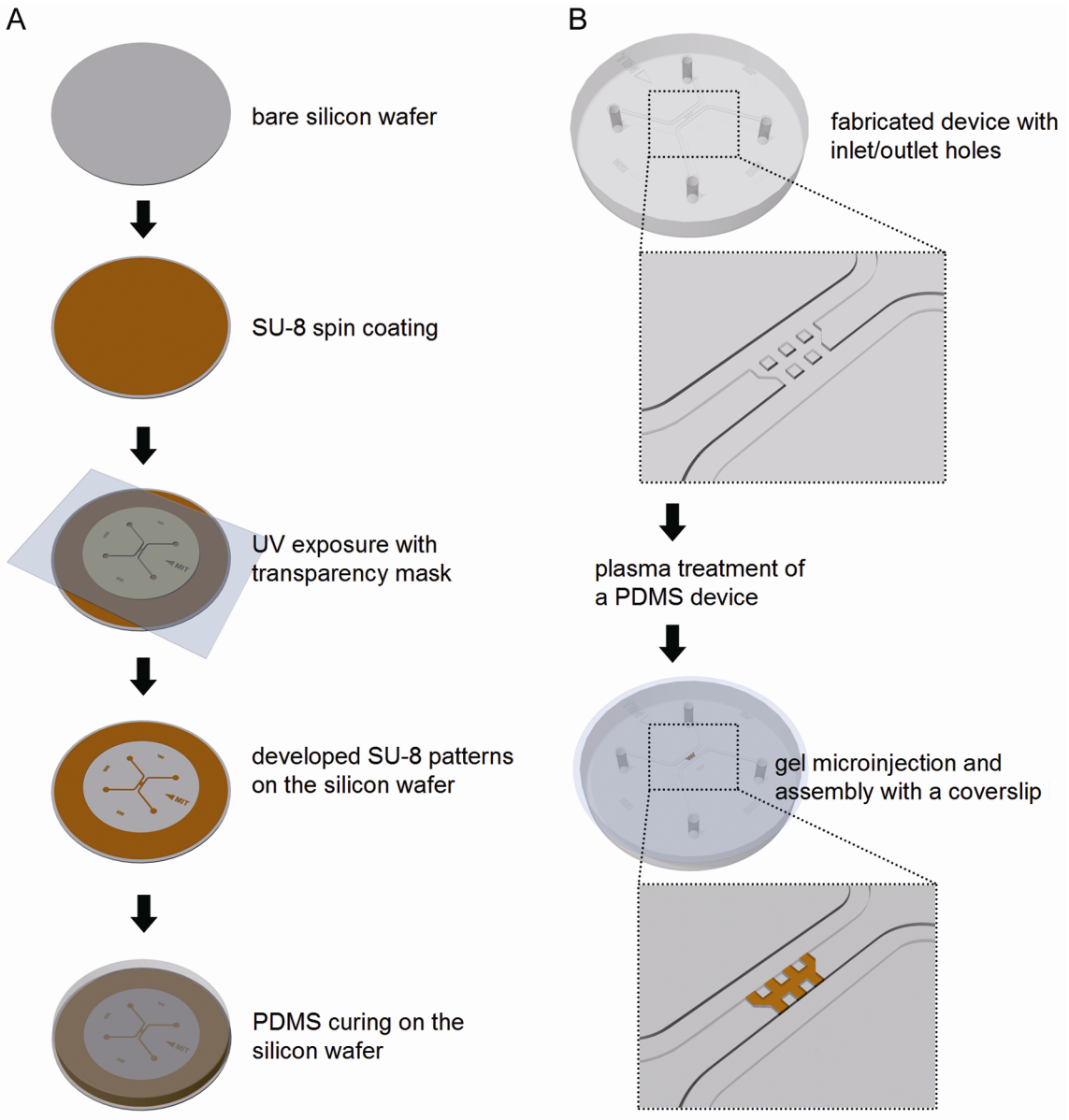
We measured the displacement of the medium level in the reservoirs during 24-h culture to be 0.583 mm (n=20, N=3). The displacement was then used to calculate *V(t=24 h)*.

The Darcy permeability was calculated by combining the displacement measurement and equation (7) and given as $K=8.9\times10^{-15}$ [m^2]. The interstitial flow velocity across the gel scaffold, *v(t)* is given as

$$v(t) = \frac{Q}{A} = \frac{\rho g K \Delta z_0}{\mu L} e^{-\frac{\rho g A K}{\mu A_r L} t} \tag{8}$$

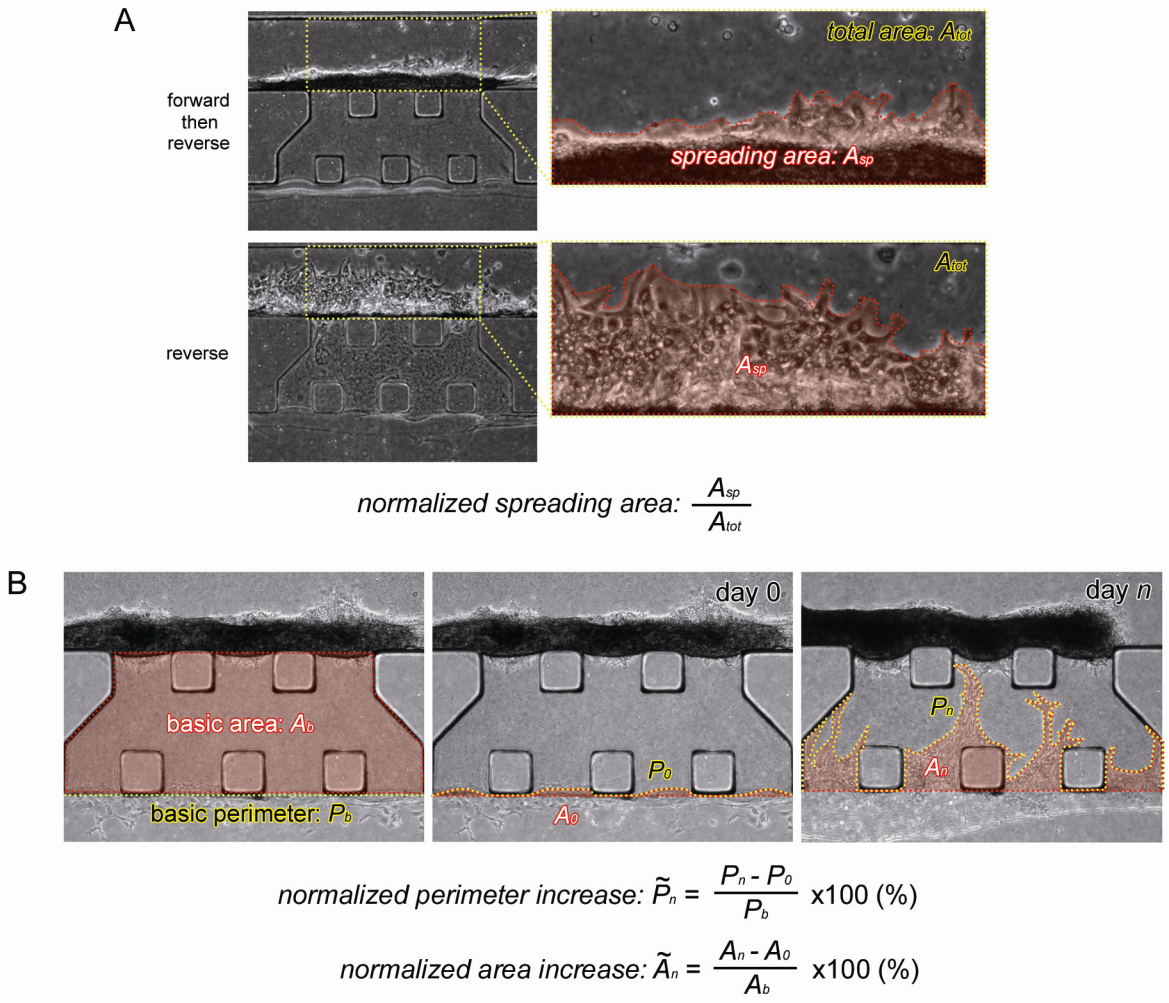
The velocity distribution was plotted using MATLAB.

SUPPLEMENTAL FIGURES

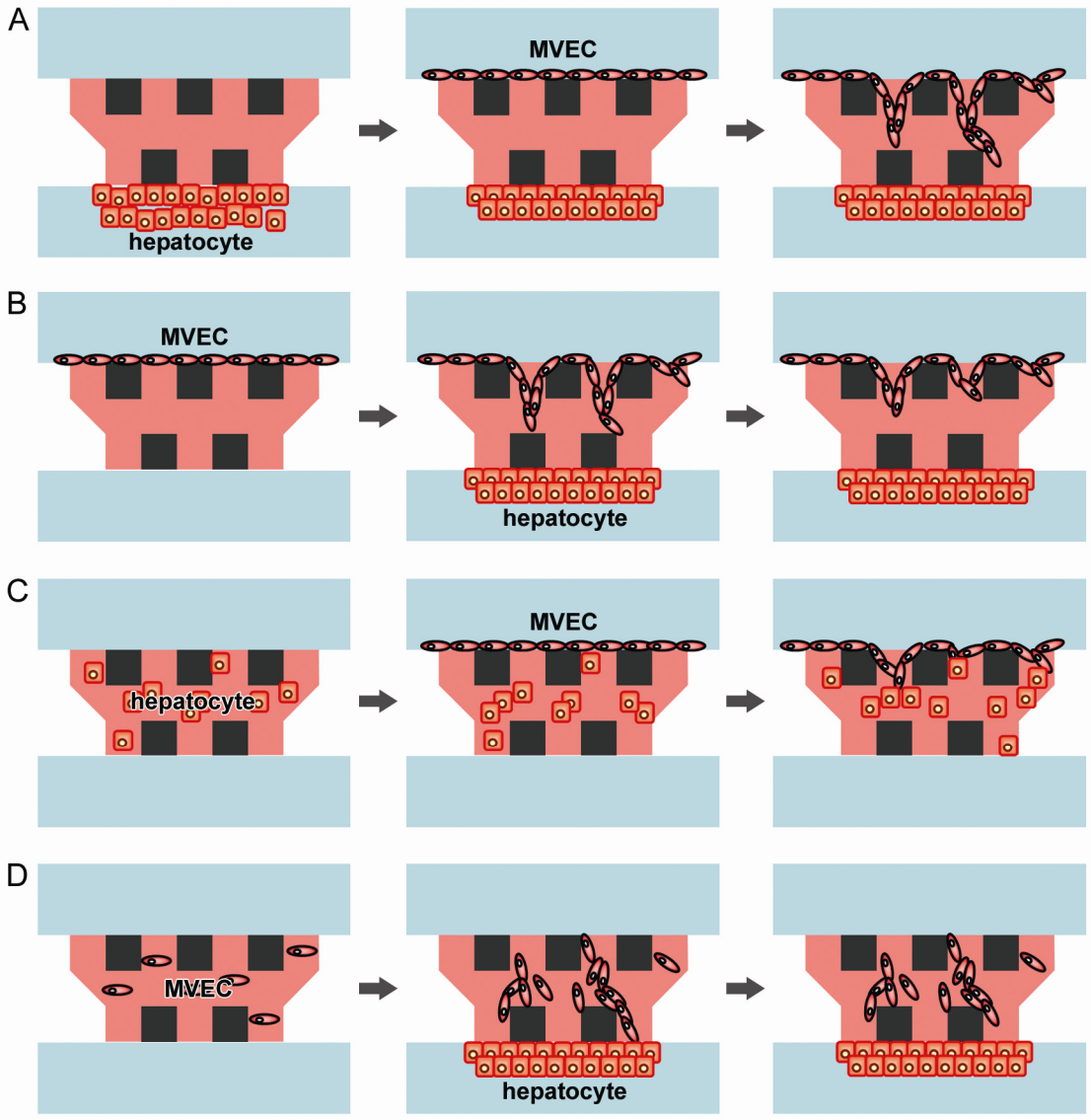


Supplemental Figure 1 Microfabrication of the microfluidic device. (A) Soft lithography process.

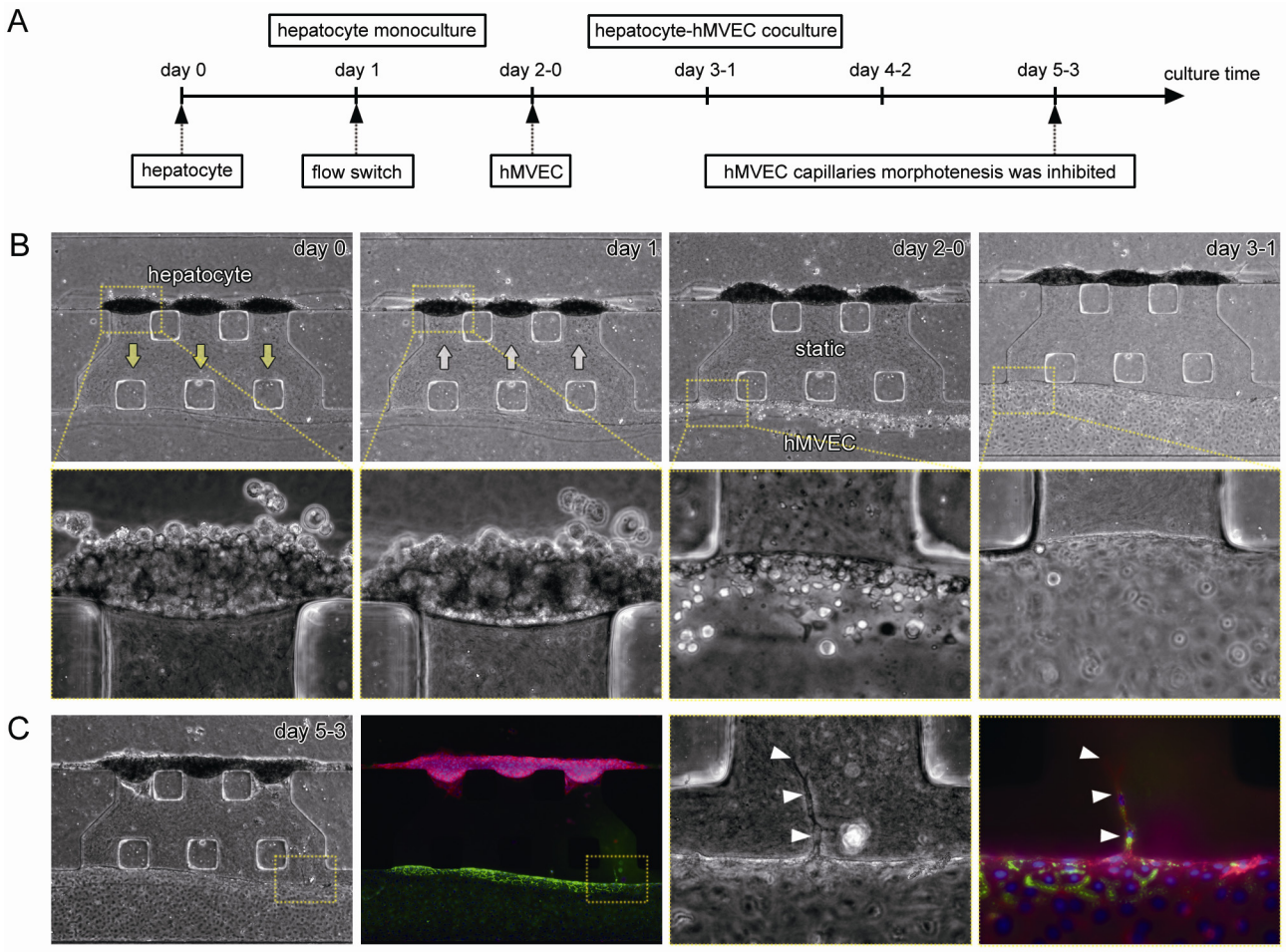
(B) Collagen gel microinjection and assembly of the PDMS device.



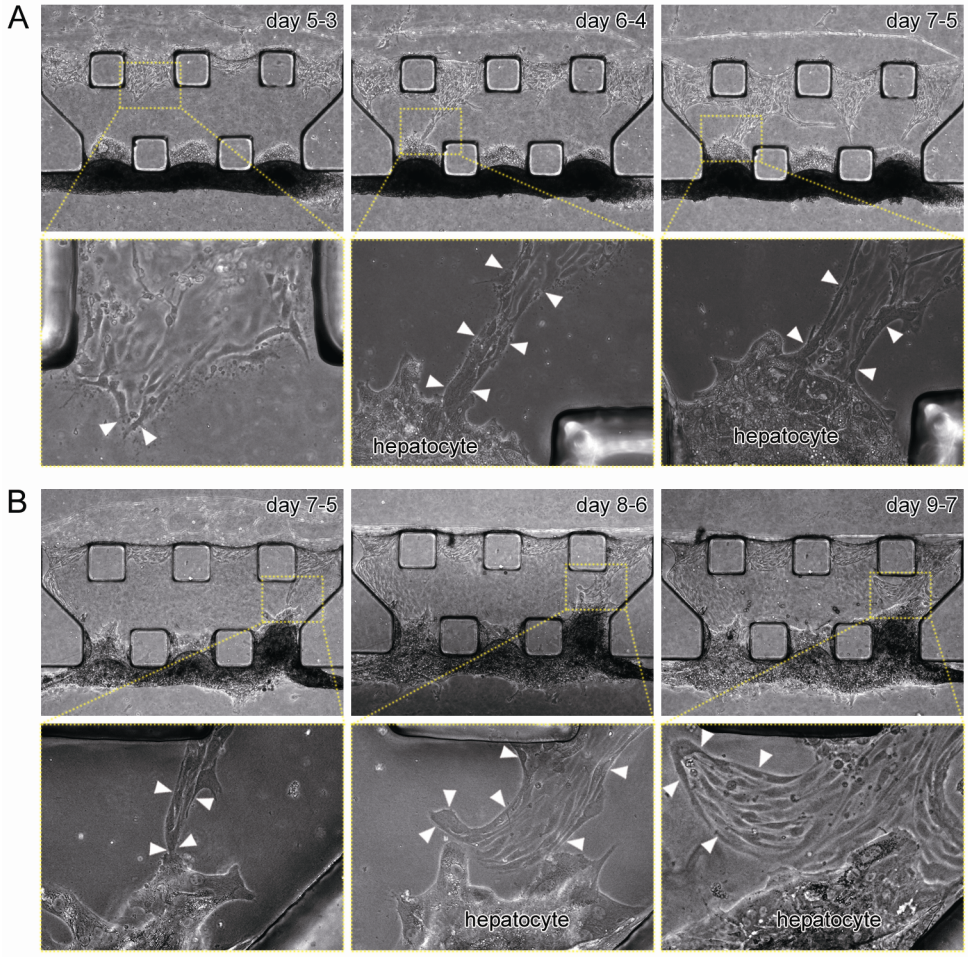
Supplemental Figure 2 Quantification of cell morphogenesis. (A) Hepatocyte morphogenesis in the presence of interstitial flow quantified by calculating "normalized spreading area". (B) Migration of rMVEC in hepatocyte-rMVEC coculture quantified by calculating "normalized perimeter increase" and "normalized area increase".



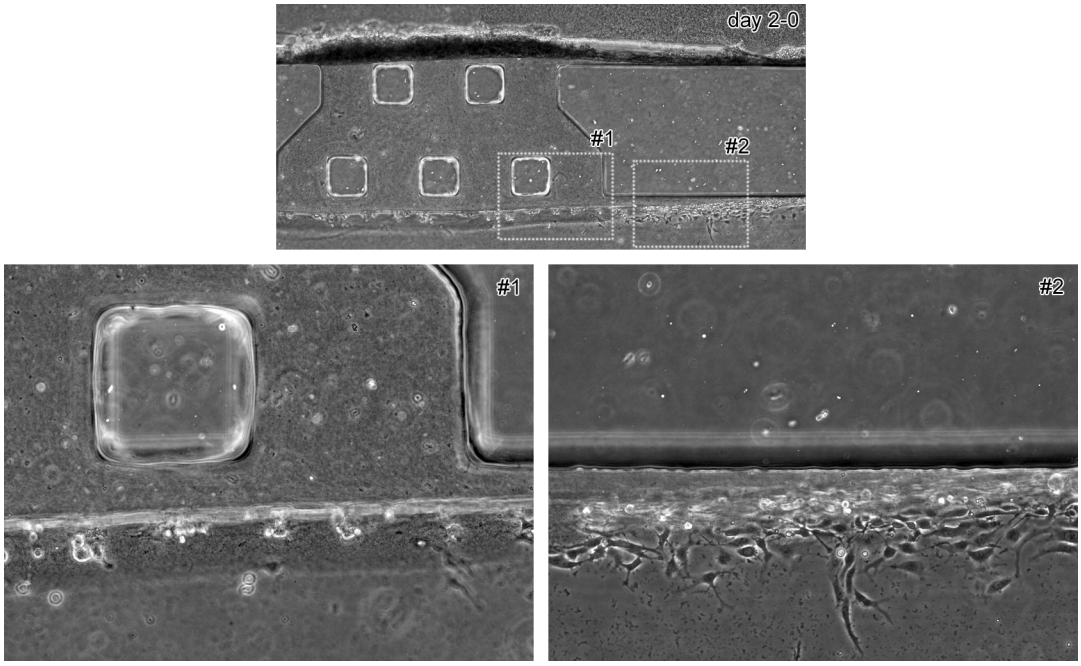
Supplemental Figure 3 Possible configurations of the hepatocyte-MVEC coculture. (A) Hepatocytes are seeded on the sidewall of a gel scaffold. After hepatocytes form 3D tissue-like structures, MVEC are added to the other side. (B) MVEC are seeded on one side. After MVEC penetrate into a gel scaffold and form capillary-like structures, hepatocytes are added to the other side. (C) Hepatocytes are embedded in a gel scaffold and MVEC are seeded on the sidewall of the gel scaffold. (D) MVEC are embedded in a gel scaffold and hepatocytes are seeded on the sidewall of the gel scaffold.



Supplemental Figure 4 Hepatocyte-hMVEC coculture. (A) Experimental protocol of the coculture. (B) Corresponding phase-contrast images of the cells. Arrows indicate the direction of interstitial flow. Hepatocytes were seeded on one side of a collagen gel scaffold. hMVEC were added to the other side on day 2. (C) The left pair represent corresponding phase-contrast and fluorescent images of cells. The cells were fixed on day 5-3 and stained for actin filaments (red), von Willebrand factor (green), and nuclei (blue). The right pair represents enlarged images of the dotted frame shown in the left pair. Arrowheads indicate a vascular sprout formed on day 5-3.



Supplemental Figure 5 rMVEC capillary-like structures in contact with hepatocyte tissue-like structures. (A) Cells cultured under static conditions. A capillary-like structure attached a hepatocyte tissue-like structure on day 6-4 (arrowheads, day 6-4). rMVEC remain attached to hepatocytes on the following day (arrowheads, day 7-5). (B) Cells cultured under forward flow, followed by reverse flow and static conditions. A capillary-like structure became attached to hepatocytes on day 7-5. The capillary-like structure deviated after attaching to the hepatocyte tissue-like structure.



Supplemental Figure 6 Phase-contrast images of the cells in hepatocyte-rMVEC coculture. Cells were photographed 5 h after rMVEC addition. rMVEC attached and spread on the hepatocyte-free portion of a collagen gel scaffold surface (#2). However, fewer rMVEC attached and spread on the gel surface that was exposed to hepatocytes (#1). Similar phenomena were also observed in hepatocyte-hMVEC coculture.