Supplementary information

SI.1: Estimation of effective culture time, critical perfusion rate and shear stress in the upper microchamber.

The effective culture time (ECT) introduced by Young and Beebe was calculated to define the maximal static cultivation time. It is the time related to proper media change interval, which should be considerable in micro-scale culture condition.

\[ D_a = \frac{K_m h \sigma}{D C_0} = \left( \frac{h^3}{D} \right) \left( \frac{C_0 h K_m \sigma}{T_d} \right) = \frac{T_d}{T_r} \]

where \( K_m \) is uptake rate [mol min \(^{-1}\)], \( h \) is chamber height [mm], \( \sigma \) is cell density [mm \(^{-2}\)], \( D \) is diffusivity [\( \mu \)m s \(^{-2}\)], \( C_0 \) is initial concentration [mol mm \(^{-3}\)], and \( T_d \) is diffusion time [min], \( T_r \) is reaction time [min].

As shown by the \( D_a \) equation, diffusion time is an order of magnitude higher than reaction time and \( h \) in upper chamber (\( h = 0.5 \) mm) is five times smaller than that in the in-well culture system (\( h = 2.5 \) mm), which is comparable to the culture flask condition.

\[ ECT = T_r = \frac{C_0 h}{K_m \sigma} \]

\( T_r \) is equivalent to ECT in diffusion-dominant system, which is linearly proportional to \( h \). HeLa cells are cultured in culture flasks with 25 cm \(^2\) in 5 ml volume of medium \( h \) is 2 mm and medium is changed every three days (72 hours). As \( h \) of the upper cell microchamber is 0.5 mm, ECT for our microfluidic chip should be 18 hours. When diffusion-based transport experiments are done, it is considered to limit the static incubation time in the chip to 18 hours.

From this concept, critical perfusion rate (CPR) was introduced, which is combined with ECT and micro cell chamber dimension.

\[ U_m = \frac{V}{T_r} \]

\[ \kappa = \frac{T_r}{T_c} = \left( \frac{C_0 h}{K_m \sigma} \right) \]

where \( U_m \) is CPR [mm\(^3\) min\(^{-1}\)], \( V \) is volume of upper cell chamber [mm\(^3\)], \( T_c \) is residence time or convection time [min], \( \kappa \) is the ratio of convection and reaction time scales.

The volume of upper cell chamber is about 205 mm\(^3\) thus CPR for our chip is considered as 0.19 \( \mu \)l min\(^{-1}\) which we keep above to avoid nutrient depletion and waste accumulation.

Shear stress is one of the considered factors when we decide the proper volumetric flow rate for perfusion to cells. Parabolic flow profile can estimate wall shear stress by the Newton’s law of viscosity in 2D Poiseuille flow systems.

\[ \tau = \frac{6 \mu Q}{h^2 w} \]

where \( \mu \) is viscosity [kg m\(^{-1}\) s\(^{-1}\)], \( Q \) is volumetric flow rate [m\(^3\) s\(^{-1}\)], \( h \) is chamber height [m], and \( w \) is chamber width [m]. Gaver and Kute developed a theoretical model of hydrodynamic shear stress on a adherent cells to microchannel wall. It is increased up to three times the surface without cells if the ratio of cell height to channel height is 0.1.

The adherent HeLa cell height practically measured by confocal microscope is around 2-10 \( \mu \)m and the channel height is 500 \( \mu \)m. Therefore, we considered the fluid stress is nearly same with that when the microchannel has no adherent cells.
Although different cell types accept a range of shear stress, most cells are resistant up to 1 dyne cm\(^{-2}\). HeLa cells even up to 4 dyne cm\(^{-2}\). When a perfusion rate of 5 and 6.8 µl min\(^{-1}\) is applied for upper cell microchamber in our microfluidic chip, wall shear stress can be estimated as 0.0013 and 0.0018 dyne cm\(^{-2}\), respectively. Therefore, the range between 5 to 6.8 µl min\(^{-1}\) that we used throughout our experiments agrees CPR (0.19 µl min\(^{-1}\)) and introduces negligible shear stress.

SI.2: Movie of the flexible exchange of two sets of gradients. BSA-FITC and BSA-tetramethylrhodamine (12.5 µg ml\(^{-1}\)) were added in two inlets of lower gradient generator at \(t = 0\) min, and figures were captured at every 5 min interval for 25 min at 40 µl min\(^{-1}\) volumetric flow rate. Then the syringe pumping was stopped, two inlets were exchanged to each other and ran for 30 min more with 5 min time interval to save the images. Each figure filtered with GFP and dsRed channel were merged at each time point and generated in moving slide by using Openlab 5.5 software.

SI.3: (A) Immunostaining of β-catenin revealed that BIO treatment induces β-catenin accumulation in nuclei (bar = 20 µm). HeLa cells were seeded on glass slides, treated with 5 µM BIO in DMSO and the solvent only and analyzed for the subcellular localization of β-catenin. (B) Western blot analysis and 2D-densitometry revealed that BIO treatment results in an increase of active β-catenin (α-ABC) but not of total β-catenin (α-β-catenin) in a dose dependent manner. The following BIO concentrations were tested: 1=0, 2=0.71, 3=1.43, 4=2.14, 5=2.86, 6=3.57, 7=4.29, 8=5.0 µM. The densitometry is obtained from the relative intensity of anti-active-β-catenin (ABC) bands to that of total β-catenin and normalized to the value of 5 µM BIO. The error bars show standard errors between two independent experiments.
SI.4: The Wnt/β-catenin signaling pathway is activated upon a certain threshold of BIO concentration and treatment time in HeLa cells cultured under static conditions in 24 microwell plates for control. BIO was treated in each well at different concentration as follows; 1=0, 2=0.29, 3=0.57, 4=0.86, 5=1.14, 6=1.43, 7=1.71, 8=2.0 µM. In each experiment three immunocytometric images were taken per each condition to count the total number of cells (N_{DAPI}) and β-catenin nuclear accumulated cells (N_{bCat}) by intensity. The average of total counted cells per each condition was 937 cells and that of cell density in each image was 1.3 x 10^5 cm^{-2}. Different treatment periods revealed the time-span between BIO application and intracellular Wnt/β-catenin signaling response. The time-span includes diffusion through the height of the microwell. The comparable tests were performed after 4 hrs and 8 hrs of BIO incubation. No activation of the Wnt signaling according our criteria was found in all different concentrations. The error bars show standard errors among three independent experiments.

SI.5: The relative number of cells with nuclear β-catenin to that of DAPI stained cells showed nearly linearly increased tendency by the channel number increase in diffusion-based transport mode. Total 2965 cells were counted from four independent microfluidic chips. Five images were taken from each channel of each chip. Forty images per chip were used for selecting the number of β-catenin nuclear accumulated cells (N_{bcat}) out of DAPI stained cells (N_{DAPI}) by intensity difference in images. Activation of Wnt/β-catenin signaling pathway by BIO is concentration-dependent in the microfluidic chip. To find out the exact threshold concentration static diffusion time should be controlled due to lateral diffusion among channels. The error bars show the standard errors among the chips.
SI.6: Fluorescent images of each channel of the bipolar Wnt3a and Dkk-1 gradient application. Channel 1 is supposed to have 500 ng ml⁻¹ concentration of Dkk1 with no Wnt3a and channel 8 contains 500 ng ml⁻¹ Wnt3a with no Dkk-1. In channel 7 and 8 accumulation of β-catenin is shown overlapped with DAPI (arrows, bar=20 μm).
References