Electronic Supplementary Material (ESI) for Lab on a Chip

ELECTRONIC SUPPLEMENTARY INFORMATION

Continuous Perfusion Microplate for Cell Culture

Vasiliy N. Goral, Chunfeng Zhou, Fang Lai and Po Ki Yuen*

Science and Technology, Corning Incorporated, Corning, New York 14831-0001

* Corning Incorporated
Science & Technology
Corning, New York 14831-0001
Phone: (607) 974-9680
Fax: (607) 974-5957
Email: yuenp@corning.com

MATHEMATICAL SIMULATION MODEL

We developed a mathematical simulation model based on Darcy's Law, which describes the flow of a fluid through a porous material when an external pressure is applied across the two ends of the porous material,[1] to predict the fluid perfusion flow dynamics in the perfusion microplate

$$Q = \frac{\kappa A_m}{\mu}\frac{\Delta P}{L} = \frac{\kappa A_m}{\mu}\frac{\rho g \Delta h}{L} = \frac{\kappa A_m}{\mu}\frac{\rho g \Delta V}{A_w L} \quad \text{(S1)}$$

In Equation (S1), Q (m^3 s^{-1}) is the fluid perfusion flow rate between two connected wells, κ (m^2 or Darcy, which is equal to 1 μm^2) is the permeability of the porous material (the cellulose membrane/filter paper). A_m (m^2) and A_w (m^2) are the cross-sectional areas of the porous material and the microplate well, respectively, μ (Pa s) is the fluid viscosity, ΔP (Pa) is the pressure drop across the porous material, L (m) is the length of the porous material, ρ (kg m^{-3}) is the fluid density, g (m s^{-2}) is the gravity constant, and Δh (m) and ΔV (m^3) are the fluid height difference and the fluid volume difference between two connected wells, respectively. Since the fluid perfusion flow rate Q affects the change of fluid height (h) or volume (V) in the two connected wells, at time dt, we obtain

$$Q\ dt = dV \quad \text{(S2)}$$

Thus, from Equation (S1) and (S2), we obtain

$$\frac{dV}{dt} = \frac{\kappa A_m}{\mu}\frac{\rho g \Delta V}{A_w L} = \frac{\Delta V}{\tau} \quad \text{(S3)}$$

where

$$\tau = \frac{\mu}{\kappa A_m}\frac{A_w L}{\rho g} \quad \text{(S4)}$$

is the characteristic fluid perfusion time of the porous material that connects the two wells. This characteristic perfusion time τ determines the fluid flow rate between the two connected wells. It takes into account the fluid properties (μ and ρ), the microplate well geometry (A_w), and the porous material dimensions (L and A_m) and permeability property (κ). In addition, all the parameters required to estimate τ are available except the permeability of the porous material κ, which was fitted experimentally. The initial guess value for κ can be obtained using the Ergun Equation for the permeability of a packed fluidized bed.[2, 3] When the fluid flow rate is small and the inertia effect is negligible, the

permeability given by the Ergun Equation can be simplified as

$$\kappa = \frac{\varepsilon^3 {d_p}^2}{150(1-\varepsilon)^2} \tag{S5}$$

where ε is the porosity of the packed fluidized bed and d_p (m) is the particle diameter. Although the porous material (open pores) is very different from the packed fluidized bed (packed spheres), Equation (S5) provides a good starting point when fitting κ to the experimental data.

Equation (S3) describes the fluid volume change as a function of time in each well, which essentially depends on the fluid volume/height difference between the two connected wells, the properties of the fluid and the porous material connecting the two wells. Thus, from Equation (S3), we obtain the equations predicting the fluid volume change in the source, the cell culture and the waste wells as a function of time

$$\begin{aligned} \frac{dV_s}{dt} &= \frac{V_{cc} - V_s}{\tau_{s-cc}} \\ \frac{dV_{cc}}{dt} &= \frac{V_s - V_{cc}}{\tau_{s-cc}} + \frac{V_w - V_{cc}}{\tau_{cc-w}} \\ \frac{dV_w}{dt} &= \frac{V_{cc} - V_w}{\tau_{cc-w}} \end{aligned} \tag{S6}$$

where V_s, V_{cc} and V_w represent the fluid volumes in the source, the cell culture and the waste wells, respectively, while $\tau_{s\text{-}cc}$ and $\tau_{cc\text{-}w}$ are the characteristic fluid perfusion times of the porous material connecting the source and the cell culture wells, and connecting the cell culture and the waste wells, respectively. We further simplify Equation (S6) when both porous materials are identical, *i.e.*, $\tau_{s\text{-}cc} = \tau_{cc\text{-}w} = \tau$ to

$$\frac{d}{dt}\begin{pmatrix} \Delta V_{s-cc} \\ \Delta V_{cc-w} \end{pmatrix} = \begin{pmatrix} -2/\tau & 1/\tau \\ 1/\tau & -2/\tau \end{pmatrix} \begin{pmatrix} \Delta V_{s-cc} \\ \Delta V_{cc-w} \end{pmatrix} \tag{S7}$$

where $\Delta V_{s\text{-}cc} = V_s - V_{cc}$ and $\Delta V_{cc\text{-}w} = V_{cc} - V_w$. Since the eigenvalues of the matrix in Equation (S7) are $-3/\tau$ and $-1/\tau$, we can solve analytically for $\Delta V_{s\text{-}cc}$ and $\Delta V_{cc\text{-}w}$

$$\begin{aligned} \Delta V_{s-cc}(t) &= a\, e^{\frac{-3t}{\tau}} + b\, e^{\frac{-t}{\tau}} \\ \Delta V_{cc-w}(t) &= -a\, e^{\frac{-3t}{\tau}} + b\, e^{\frac{-t}{\tau}} \end{aligned} \tag{S8}$$

where $\boldsymbol{a} = ½\,(V_s - 2\,V_{cc} + V_w)$ and $\boldsymbol{b} = ½\,(V_s - V_w)$ at $t = 0$, which can be determined by

the initial fluid volumes of the source, the cell culture and the waste wells. Finally, we solve for $V_s(t)$, $V_{cc}(t)$ and $V_w(t)$ from Equations (S8) and (S6) by considering total mass conservation such that $dV_s + dV_{cc} + dV_w = 0$

$$\begin{pmatrix} V_s(t) \\ V_{cc}(t) \\ V_w(t) \end{pmatrix} = \begin{pmatrix} \frac{1}{3} & \frac{2}{3} & \frac{1}{3} \\ \frac{1}{3} & -\frac{1}{3} & \frac{1}{3} \\ \frac{1}{3} & -\frac{1}{3} & -\frac{2}{3} \end{pmatrix} \begin{pmatrix} V_s(0) + V_{cc}(0) + V_w(0) \\ a\, e^{\frac{-3t}{\tau}} + b\, e^{\frac{-t}{\tau}} \\ -a\, e^{\frac{-3t}{\tau}} + b\, e^{\frac{-t}{\tau}} \end{pmatrix} \quad \text{(S9)}$$

Therefore, we use Equation (S9) with the fitted κ value to predict the fluid perfusion flow dynamics in the source, the cell culture and the waste wells when they are connected by two identical porous materials.

MATERIALS AND METHODS

Perfusion microplate assembly and sterilization

First, we cut 96 holes (6.5 mm in diameter and 9 mm apart) and 64 channels (1 mm wide) connecting two holes in a set of three holes in a 110 mm × 75 mm double-sided pressure sensitive adhesive (PSA) sheet (ARcare® 90106, Adhesives Research, Inc., Glen Rock, PA, USA) using a desktop digital craft cutter (Fig. S1).[4] Next, we cut 1 mm wide and 3.5 mm long pieces of a cellulose membrane with either a 1.2 µm (Whatman® ST 69, Catalog No. 10403012, Whatman GmbH, Dassel, Germany), 5 µm (Order No. 12342-47-K, Sartorius Stedim Biotech GmbH, Göttingen, Germany) or 8 µm (Whatman® AE 99, Catalog No. 10400112, Whatman) pore size from a 47 mm diameter cellulose membrane using an X-Acto® precision knife (Elmer's Products, Inc., Westerville, OH, USA). In some cell experiments, instead of cellulose membranes, pieces of 300 – 600 µm wide, 115 µm thick and 3.5 mm long filter paper strips were cut from a 110 mm diameter filter paper (Grade 50, Catalog No. 1450-110, Whatman) and tested as an alternative porous material. We started the assembly process by peeling off the first protective layer and exposing the first PSA surface of the cut double-sided PSA sheet before attaching it to a polystyrene film, which was taken from a Corning® HYPERStack™ Cell Culture Vessel (Corning Incorporated, Corning, NY, USA) (Fig. 1S). After that, we aligned and inserted the pieces of cellulose membranes/filter papers into the cut channels of the cut double-

sided PSA sheet. In order to complete the assembly process, we peeled off the second protective layer and exposing the second PSA surface of the cut double-sided PSA sheet with the inserted cellulose membranes/filter papers. We then aligned and attached the bottom of a standard flat bottom 96-well holey (bottomless) microplate (Corning Incorporated) to the second PSA surface. Care was taken to ensure that the cut double-sided PSA sheet and the polystyrene film were fully adhered to the edges of each well bottom without any air bubbles. Also, each individual cellulose membrane/filter paper was inspected under a microscope to ensure that there were no gaps formed between the two long edges of the cellulose membrane/filter paper and the cut channel edges in the cut double-sided PSA sheet. Thus, after assembly and inspection, each group of three wells should be fluidically linked together on the bottom of the wells by the two cellulose membranes/filter papers once liquid was pipetted into the three wells. Finally, before each cell experiment, we sterilized each well of the 96-well perfusion microplate with 300 µl of 70 % ethanol for 30 minutes, washed twice with 300 µl of deionized water and air dried the assembled microplates in a sterile cell culture hood for 15 minutes.

For conventional 96-well microplate assembly, we attached the polystyrene film to the bottom of the standard flat bottom 96-well holey microplate by using the same double-sided PSA sheet with the 96 cut holes. We also used the same sterilization protocol for the conventional 96-well microplates.

Perfusion flow characterization

After developing a mathematical simulation model, we experimentally characterized the fluid perfusion flow dynamics (volume as a function of time) between the three connected wells of the 96-well perfusion microplate with a carboxyfluorescein fluorescent dye (8×10^{-5} M) (Catalog No. 54115, Sigma-Aldrich, St. Louis, MO, USA) in phosphate buffered saline (PBS) solution using a microplate reader (VICTOR3™ 1420 Multilabel Counter, PerkinElmer, Inc., Waltham, MA, USA) at room temperature (Fig. S2). In order to accurately monitor the fluid volumes in the three connected wells, we first obtained a fluorescence signal calibration curve as a function of fluorescent dye solution volume in the well of a conventional 96-well microplate at room temperature using the microplate reader. Then, 220 µl, 120 µl and 20 µl or 290 µl, 50 µl and 20 µl

aliquots of the fluorescent dye solution were separately pipetted into the source, the cell culture and the waste wells, respectively, and the fluorescent signal inside the wells was monitored in real-time by the microplate reader.

Long-term proliferation of human hepatocyte tumor cell line (C3A cells) when cultured in perfusion microplate

We first thawed and cultured cryopreserved C3A cells, a derivative of HepG2/C3A human hepatoblastoma cell line (CRL-10741™, American Type Culture Collection (ATCC), Manassas, VA, USA), in a sterile cell culture flask (Product # 430641, Corning Incorporated) in Eagle's Minimum Essential Medium (EMEM) (ATCC® No. 30-2003, ATCC) supplemented with 10 % fetal bovine serum (FBS) (Catalog No. 16000-077, Invitrogen Corporation, Carlsbad, CA, USA) and 1 % Penicillin-Streptomycin (Catalog No. 15140-163, Invitrogen Corporation) in a CO_2 HEPA incubator (Model 3130, Forma Scientific, Inc., Marietta, OH, USA) at 37 °C, 95 % humidity and 5 % CO_2. Next, we seeded the C3A cells (50 k / well) from the cell culture flask into individual cell culture wells of the conventional and perfusion microplates in 120 µl of Minimum Essential Medium (MEM) (Catalog No. 41090-036, Invitrogen Corporation) and incubated the microplates at 37 °C, 95 % humidity and 5 % CO_2 (Fig. S3). For the conventional 96-well microplate-based static cell culture experiments, we exchanged 100 µl out of the 120 µl of medium daily in the cell culture wells in one set of the experiments while no medium exchange was performed in the other set of the experiments. For the 96-well perfusion microplate-based perfusion cell culture experiments, we started the fluid perfusion after 24 hours of cell seeding with 220 µl of MEM, 120 µl of MEM with C3A cells and 20 µl of MEM in the source, the cell culture and the waste wells, respectively. If the volumes in all three wells reached equilibrium before the experiments ended, the volumes in the source and the waste wells were reset to the initial volumes of 220 µl and 20 µl, respectively. No manual medium exchange was performed in the cell culture wells throughout the experiments. After 4 days of cell culture, the LIVE/DEAD® Viability/Cytotoxicity Assay Kit and PrestoBlue® Cell Viability Reagent were performed to determine C3A cell viability and to quantitatively measure the proliferation of C3A cells, respectively.

LADMAC cell culture

LADMAC cells, a transformed cell line derived by transfecting mouse bone marrow cells highly enriched for macrophage progenitors after transfection with human cellular myc-homologous DNA sequences in the pBR325 plasmid (pR myc), secrete the growth factor colony stimulating factor 1 (CSF-1). CSF-1 is capable of supporting the *in vitro* proliferation of mouse bone marrow macrophages.[5] The LADMAC cell line (CRL-2420™, ATCC) is used to produce LADMAC conditioned medium containing CSF-1 which will support the growth of the macrophage cell lines EOC 2 (CRL-2467™, ATCC), EOC 13.31 (CRL-2468™, ATCC), EOC 20 (CRL-2469™, ATCC), I-11.15 (CRL-2470™, ATCC) and I-13.35 (CRL-2471™, ATCC). We first thawed and cultured cryopreserved LADMAC cells in a sterile cell culture flask using EMEM supplemented with 10 % FBS at 37 ºC, 95 % humidity and 5 % CO_2. We manually exchanged the medium every 2 to 3 days in the cell culture flask to promote the LADMAC cell growth.

LADMAC conditioned medium containing CSF-1

The LADMAC conditioned medium containing CSF-1 was prepared from the LADMAC cells. We first cultured LADMAC cells to confluence in the cell culture flask as described above. After 5 to 7 days of cell culture, we collected the medium (supernatant) from the cell culture flask and centrifuged (Eppendorf® Centrifuge 5810R, Eppendorf AG, Hamburg, Germany) it at 125 ×g for 5 to 10 minutes. Next, we filtered the centrifuged medium using a 0.22 µm filter (Corning® 430767, Corning Incorporated). Finally, the filtered medium was stored at -20 ºC for later use.

EOC 20 cell culture

EOC 20 cells are an immortalized cell line derived from the brain of an apparently normal 10 day old mouse.[6] The EOC 20 cells depend on the growth factor CSF-1, which is secreted by the LADMAC cells, for growth. Before microplate-based cell culture experiments, we first thawed and cultured cryopreserved EOC 20 cells in a sterile cell culture flask using Dulbecco's Modified Eagle's Medium (DMEM) with 4 mM L-glutamine adjusted to contain 4.5 g/L glucose, 1.5 g/L sodium bicarbonate and 1.0 mM sodium pyruvate (ATCC® No. 30-2002, ATCC) supplemented with 10 % FBS and 20 % LADMAC conditioned medium containing CSF-1 at 37 ºC, 95 % humidity and 5 % CO_2.

For the static cell culture experiments in the conventional 96-well microplate, we seeded the EOC 20 cells (40 k / well) from the cell culture flask into individual wells in 120 µl of DMEM supplemented with 10 % FBS both with and without 20 % LADMAC conditioned medium containing CSF-1. The conventional microplate was then incubated at 37 °C, 95 % humidity and 5 % CO_2. Next, we exchanged 100 µl out of the 120 µl of medium daily with or without supplementing it with the 20 % LADMAC conditioned medium containing CSF-1. For the 96-well perfusion microplate-based perfusion cell culture experiments, we first seeded some source wells with the LADMAC cells (20 k / well) in 120 µl of EMEM supplemented with 10 % FBS. After 24 hours of static cell culture at 37 °C, 95 % humidity and 5 % CO_2, we seeded each individual cell culture well with the EOC 20 cells (40 k / well) in 120 µl of DMEM supplemented with 10 % FBS. After 24 hours of EOC 20 cell seeding, we started the perfusion cell culture by filling the source well with either 220 µl of DMEM supplemented with 10 % FBS (without LADMAC cells) or 220 µl of EMEM supplemented with 10 % FBS (with LADMAC cells) (Fig. S4). The waste well of the perfusion microplate was filled with 20 µl of DMEM. The perfusion microplate was then incubated at 37 °C, 95 % humidity and 5 % CO_2 for 3 days without any user intervention. After 3 days of static and perfusion cell culture, we performed cell viability assays to determine EOC 20 cell viability.

Use of perfusion microplate for cell-mediated toxicity assays

We thawed and cultured cryopreserved HCT 116 colon cancer cells (CCL-247™, ATCC) in a sterile cell culture flask in MFE medium supplemented with 10 % FBS at 37 °C, 95 % humidity and 5 % CO_2. In the first set of experiments, we seeded the HCT 116 colon cancer cells (17 k / well) from the cell culture flask into individual cell culture wells of the 96-well perfusion microplate in 120 µl of MFE medium supplemented with 10 % FBS (Fig. S5a). We also filled the source and the waste wells with 120 µl of MFE medium and 120 µl of PBS buffer, respectively so that no fluid perfusion between wells would occur. After 24 hours of static cell culture at 37 °C, 95 % humidity and 5 % CO_2, which allowed attachment of the HCT 116 colon cancer cells, we exchanged 120 µl of MFE medium in the source well with 220 µl of MFE medium supplemented with either 40 µg /ml of the chemotherapeutic fluorouracil prodrug tegafur or 5-fluorouracil (5-FU)

and withdrew 100 µl of PBS buffer from the waste well to start the fluid perfusion between the source and the cell culture wells, and between the cell culture and the waste wells. After 3 days of perfusion cell culture at 37 °C, 95 % humidity and 5 % CO_2, and without any user intervention, we performed cell viability assays to determine HCT 116 colon cancer cell viability.

In the second set of experiments, we first thawed and seeded cryopreserved primary human hepatocytes (50 k / well) (Lot No. Hu4175, Invitrogen Corporation) into individual source wells of the 96-well perfusion microplate in 120 µl of MFE medium supplemented with 10 % FBS (Fig. S5b). The cell culture and the waste wells were both filled with 120 µl of PBS buffer so that no fluid perfusion between wells would occur. Then, after 1 day of static cell culture at 37 °C, 95 % humidity and 5 % CO_2, which allowed attachment of the primary human hepatocytes, we performed a Matrigel™ (Becton Dickinson, Bedford, MA, USA) overlay in the source well to establish primary human hepatocytes' polarity and restore their phenotype specific functionality. This was accomplished by exchanging the medium in the source well with the same volume of diluted Matrigel™ in serum free MFE medium (1 : 20). Next, after 2 days of static cell culture of the primary human hepatocytes at 37 °C, 95 % humidity and 5 % CO_2, we followed the same protocol as the first set of experiments to seed the HCT 116 colon cancer cells and established perfusion cell culture of the HCT 116 colon cancer cells in the cell culture well while primary human hepatocytes were cultured in the source well at the same time.

LIVE/DEAD® Viability/Cytotoxicity Assay Kit

We used the LIVE/DEAD® Viability/Cytotoxicity Assay Kit for mammalian cells (Molecular Probes, Inc., Eugene, OR, USA) to determine viability of the cultured cells following the standard protocol. Briefly, the fluorescent dye mixture was pipetted into the cell culture wells followed by the PBS buffer wash. Fluorescent live and dead staining images were collected using a Zeiss Axiovert 200 inverted fluorescence microscope equipped with an epifluorescence condenser and camera system (Carl Zeiss MicroImaging, Inc., Thornwood, NY, USA).

PrestoBlue® Cell Viability Reagent

We used PrestoBlue® Cell Viability Reagent (Catalog No. A-13261, Life Technologies Corporation, Carlsbad, CA, USA) to quantitatively measure the proliferation of cells. Briefly, a mixture of 90 µl of cell culture media and 10 µl of PrestoBlue® Cell Viability Reagent were added to each cell culture well. After incubating in the cell culture incubator for 20 minutes, 90 µl of reagent mixture was transferred from the cell culture well into a well of a 96-well black polystyrene clear flat bottom plate (Corning Incorporated). Fluorescence signal was measured using the VICTOR™ X4 Multilabel Plate Reader (Product No. 2030, PerkinElmer, Waltham, MA, USA) (Fig. S6).

SUPPLEMENTARY REFERENCES

1. O. van Genabeek and D. H. Rothman, Macroscopic manifestations of microscopic flows through porous media: phenomenology from simulation, *Annu. Rev. Eath. Planet. Sci.*, 1996, **24**, 63-87.
2. I. F. Macdonald, M. S. El-Sayed, K. Mow and F. A. L. Dullien, Flow through porous media – the Ergun equation revisited, *Ind. Eng. Chem. Fundam.*, 1979, **18**, 199-208.
3. M. R. Rezaee, A. Jafari and E. Kazemzadeh, Relationships between permeability, porosity and pore throat size in carbonate rocks using regression analysis and neural networks, J. *Geophys. Eng.*, 2006, **3**, 370-376.
4. P. K. Yuen and V. N. Goral, Low-cost rapid prototyping of flexible microfluidic devices using a desktop digital craft cutter, *Lab Chip*, 2010, **10**, 384-387.
5. M. D. Sklar, A. Tereba, B. D. Chen and W. S. Walker, Transformation of mouse bone marrow cells by transfection with a human oncogene related to c-myc is associated with the endogenous production of macrophage colony stimulating factor 1, *J. Cell Physiol.*, 1985, **125**, 403-412.
6. W. S. Walker, J. Gatewood, E. Olivas, D. Askew and C. E. Havenith, Mouse microglial cell lines differing in constitutive and interferon-gamma-inducible antigen-presenting activities for naive and memory CD4+ and CD8+ T cells, *J. Neuroimmunol.*, 1995, **63**, 163-174.

SUPPLEMENTARY FIGURES

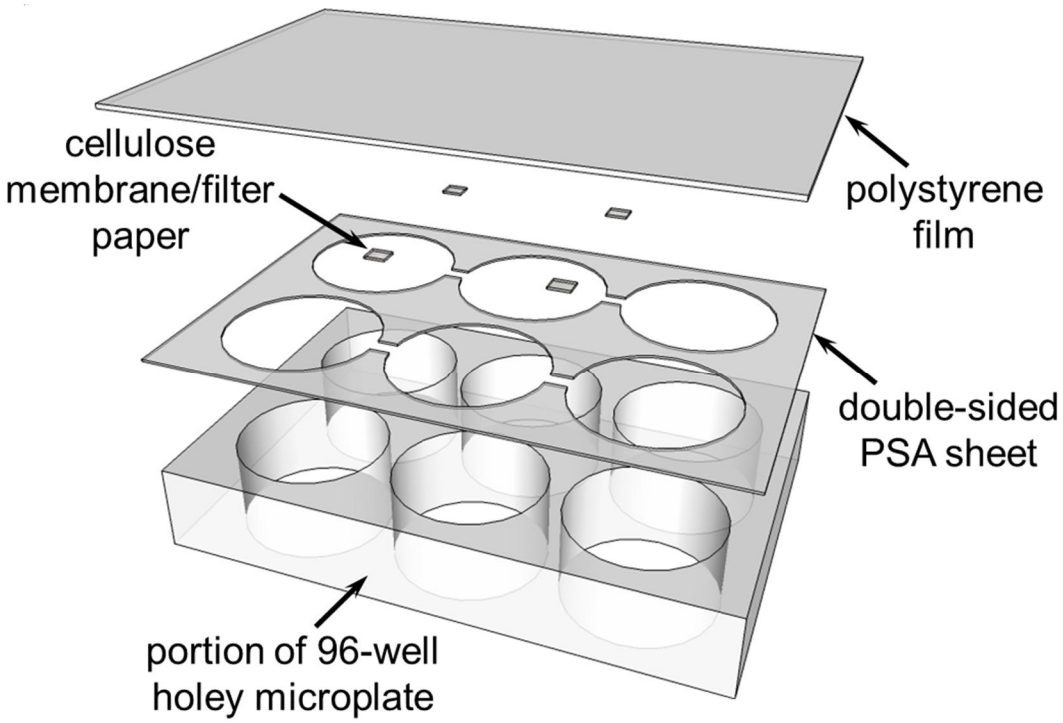

Fig. S1 Schematic diagram depicting an exploded bottom view of a portion of the 96-well perfusion microplate.

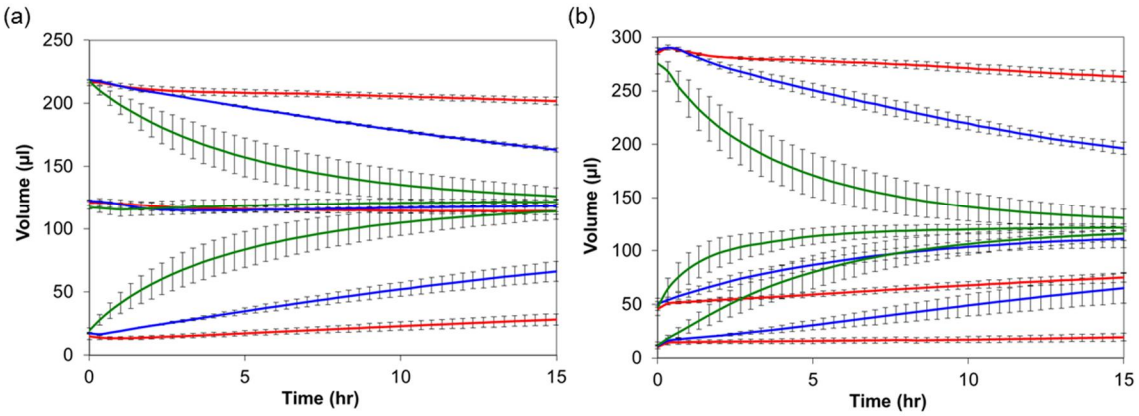

Fig. S2 Experimental fluid volumes (with error bars) in the source, the cell culture and the waste wells of the 96-well perfusion microplate with 1.2 µm (red), 5 µm (blue) or 8 µm (green) pore size cellulose membranes as a function of time. Initial fluid volumes in the source, the cell culture and the waste wells were (a) 220 µl, 120 µl and 20 µl or (b) 290 µl, 50 µl and 20 µl, respectively.

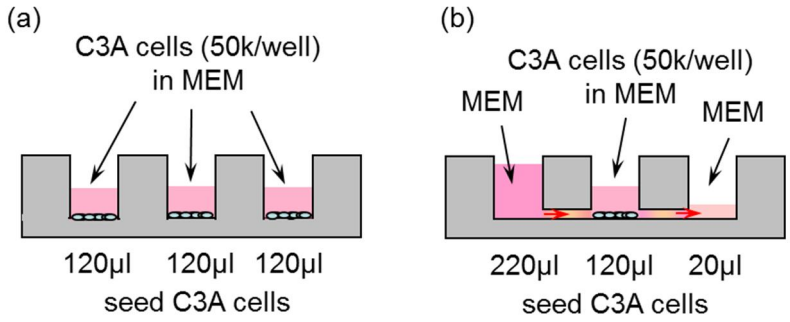

Fig. S3 Schematic diagrams of hepatocytes (C3A cells) culture in (a) the conventional 96-well microplate and (b) the 96-well perfusion microplate.

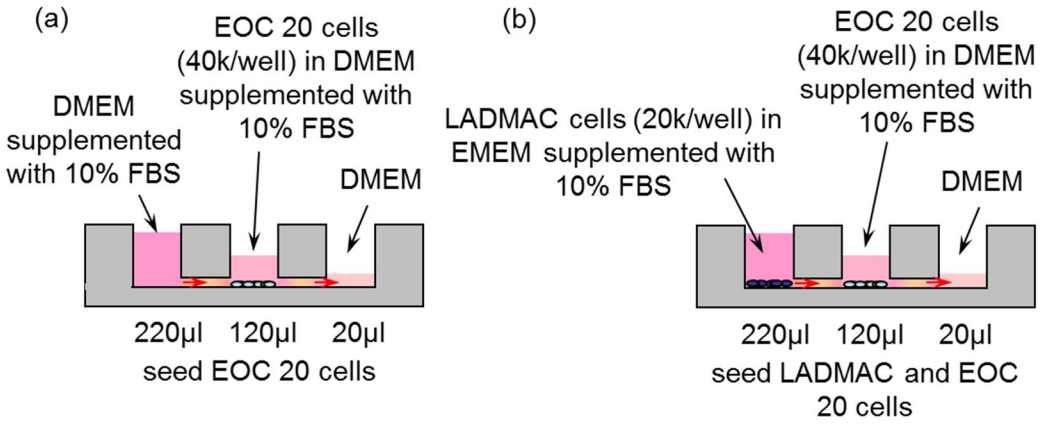

Fig. S4 Schematic diagrams of EOC 20 cells cultured in the 96-well perfusion microplate. (a) Without and (b) with the presence of LADMAC cells in the source well.

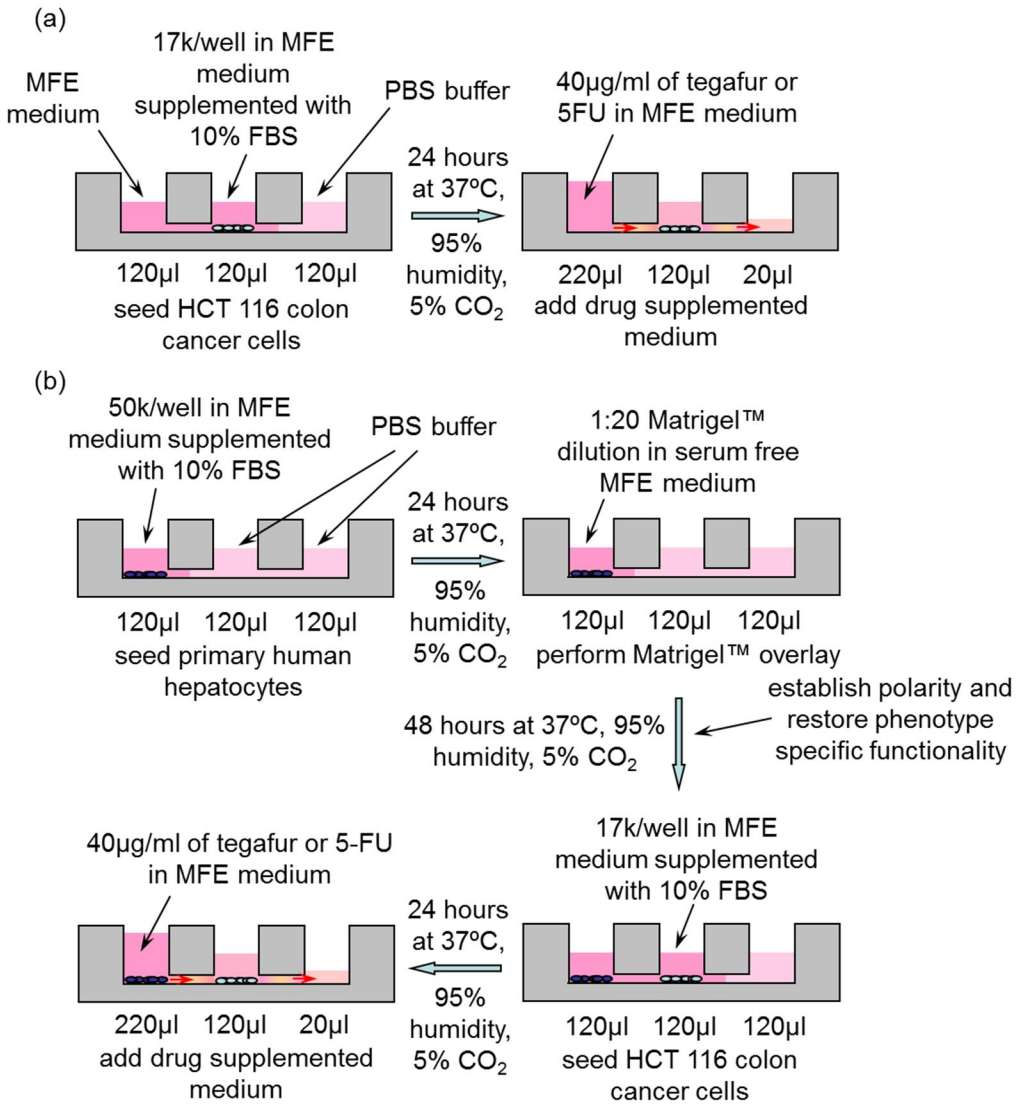

Fig. S5 Schematic diagrams of the use of the 96-well perfusion microplate for cell-mediated toxicity assays using the prodrug tegafur toward HCT 116 colon cancer cells. (a) Without and (b) with the presence of primary human hepatocytes in the source well.

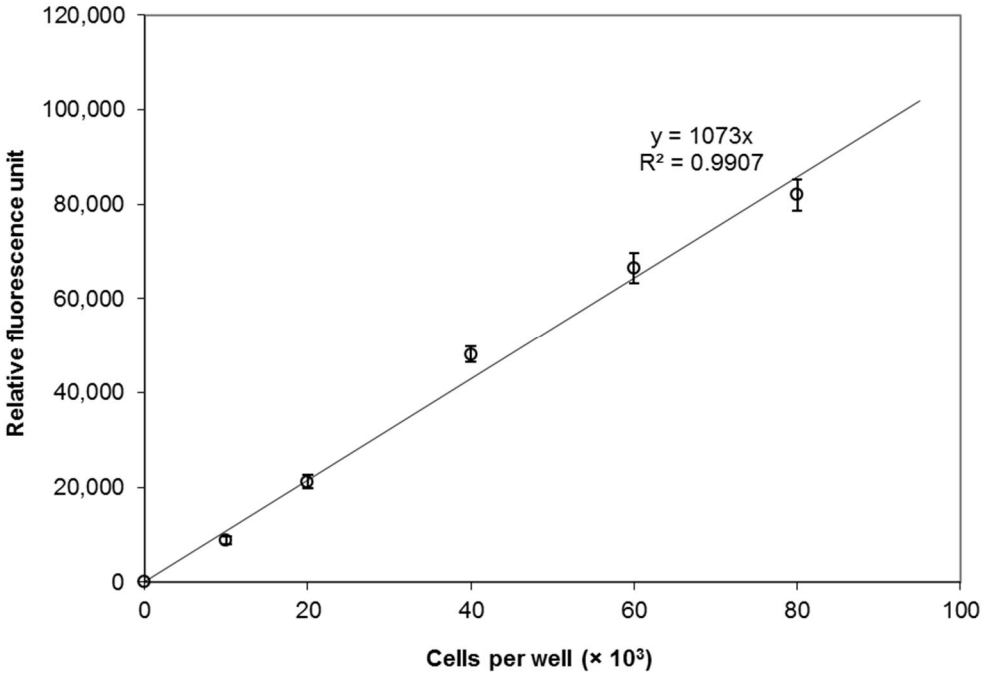

Fig. S6 Relative fluorescence unit as a function of cells per well (× 10^3) using PrestoBlue® Cell Viability Reagent with 20 minutes of incubation time.

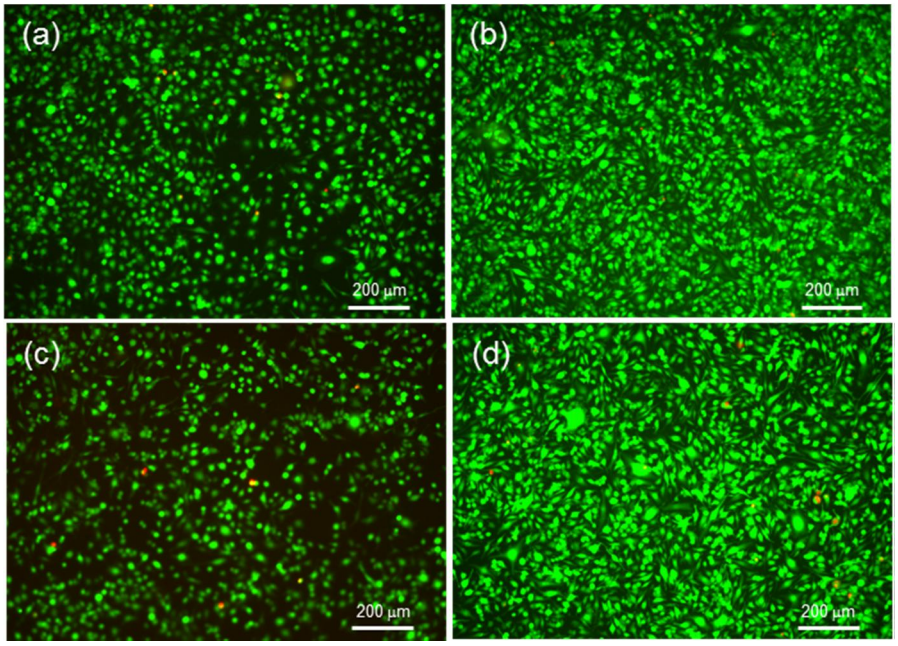

Fig. S7 Live (green)/dead (red) cell staining of EOC 20 cells after 3 days of cell culture. Static cell culture in the conventional 96-well microplate was performed with daily exchanges of media (a) without and (b) with supplementing 20 % LADMAC conditioned medium containing CSF-1. Perfusion cell culture in the cell culture well of the 96-well perfusion microplate was performed (c) without and (d) with the presence of LADMAC cells in the source well. Cell seeding density for LADMAC and EOC 20 cells was 20 k / well and 40 k / well, respectively. The fluid volume in the conventional microplate well was 120 µl. Initial fluid volumes of the source, the cell culture and the waste wells in the perfusion microplate were 220 µl, 120 µl and 20 µl, respectively. Dimensions of the filter papers were 590 µm wide and 3.5 mm long.

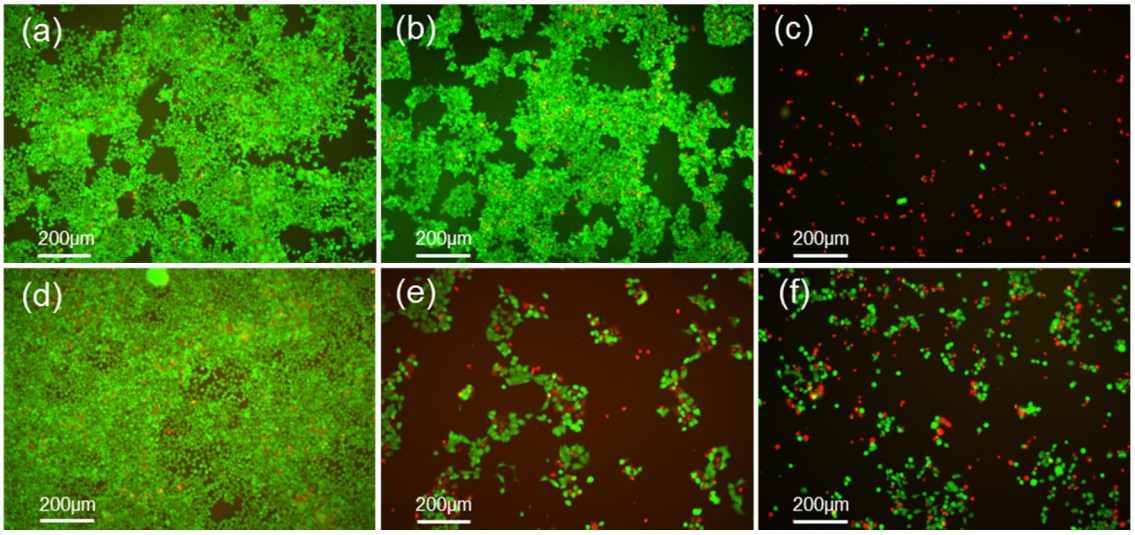

Fig. S8 Live (green)/dead (red) cell staining of HCT 116 colon cancer cells after 3 days of perfusion cell culture in the cell culture wells of the 96-well perfusion microplate with 1 mm wide, 3.5 mm long and 5 μm pore size cellulose membranes. Perfusion cell culture (a) – (c) without and (d) – (f) with the presence of primary human hepatocytes in the source well. Source well contained either 220 μl of (a) and (d) medium, (b) and (e) medium with 40 μg / ml of tegafur, or (c) and (f) medium with 40 μg /ml of 5-FU at the beginning of the perfusion cell culture. Cell seeding density for primary human hepatocytes and HCT 116 colon cancer cells was 50 k / well and 17 k / well, respectively. Initial fluid volumes of the source, the cell culture and the waste wells were 220 μl, 120 μl and 20 μl, respectively.