Electronic Supplementary Material (ESI) for Lab on a Chip

Oxygen Levels in Thermoplastic Microfluidic Devices during Cell Culture

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Electronic Supplementary Information (ESI)

Additional Figures

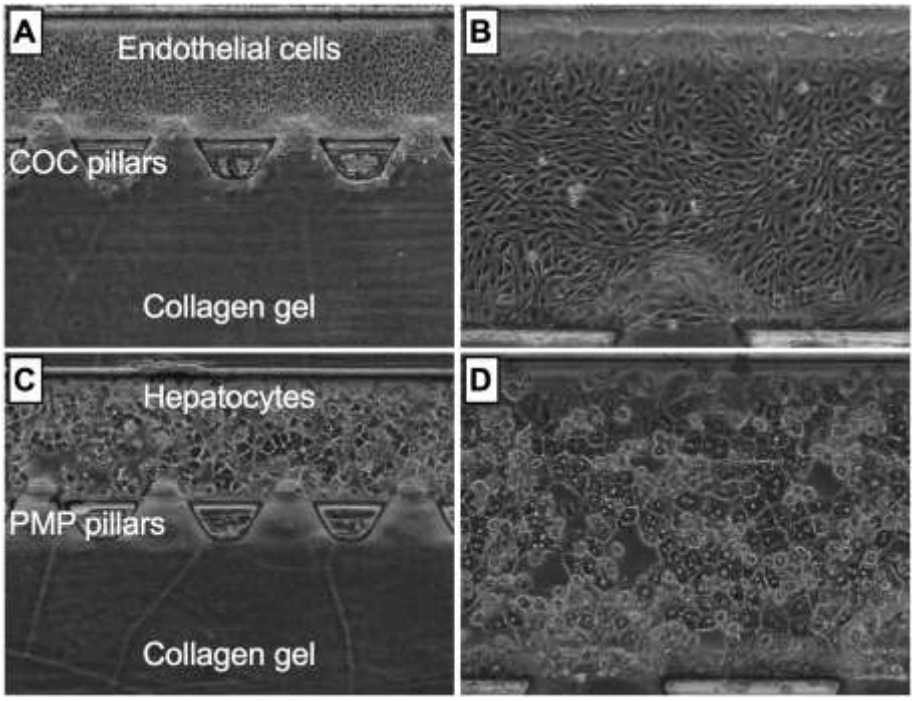


Figure S1. Phase contrast images of endothelial cells cultured in cyclic olefin copolymer (COC, A+B) or Hepatocytes in poly(methyl pentene) devices (PMP, C+D) bonded to glass coverslips. Magnifications are 4x (A + C) and 10x (B + D), respectively.

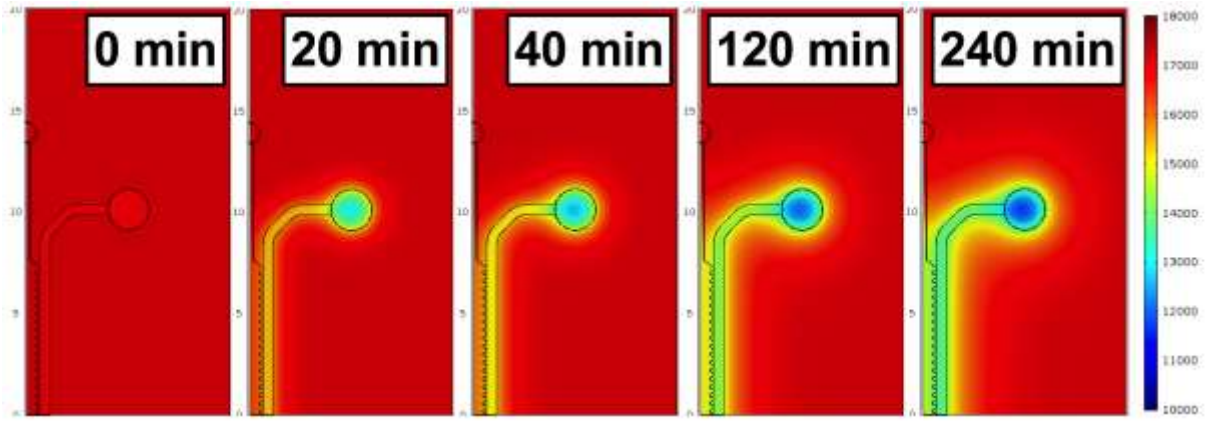


Figure S2. Heat maps of oxygen levels in a PMP device during Hepatocytes culture after indicated time periods.

S1. Details of microfluidic chip fabrication

Unless otherwise indicated, chemicals and cell culture media were purchased from Sigma-Aldrich (St. Louis, MO, USA). The microfluidic channel height was 150 μm and the total device height of 2 mm identical to that of the thermoplastic chips. PDMS was used at 10:1 ratio of base:curing agent (Sylgard 184, Dow Chemical, MI, USA). Injection-molded COC and PMP chips were prototype designs kindly provided by AIM Biotech Pte. Ltd. (Singapore). Oxygen-sensitive foils (Visisens, Regensburg, Germany) were bonded to the bottom of the devices using the following protocol (Fig. 1B): 1) removal of the protective film; 2) spin-coating with degassed PDMS at 2500 rpm for 5 min; 3) curing at 80°C for 2 h. Prior to bonding with the sensor foils, COC devices were activated by plasma treatment for 1 min and exposed to an aqueous solution of 1% v/v aminopropyltriethoxysilane (APTES) for 20 min, rinsed with distilled water and dried with compressed air.[1] The PDMS-coated foils and the patterned side of the PDMS microfluidic chips were plasma-treated for 2 min, clamped together and cured at 80°C for 2 h. To facilitate cell adhesion, devices were coated with poly-*D*-lysine (1 mg ml^{-1} in distilled H_2O) for 4 h, washed 3 times with water, and dried overnight. A 2.5 mg ml^{-1} type-I collagen gel was introduced into the device gel region, and polymerized for 30 min at 37°C. Immediately prior to cell seeding, the media channels were filled with a 50 ng ml^{-1} aqueous solution of fibronectin for 4 h.

S2. Cell culture procedures

Rat lung microvascular endothelial cells (EC) and rat hepatocytes (HEP) were used in the present study. ECs were cultured in MCDB-131 complete medium (VEC Technologies, Rensselaer, NY, USA). HEP were isolated from rats by a two-step collagenase perfusion,[2] and cultured in hepatocyte growth medium (HGM)[3] with the following modifications: 0.305 g l^{-1} niacinamide; 2 g l^{-1} d-glucose; 1 mM *L*-glutamine; 54.4 g l^{-1} $ZnCl_2$; 75 g l^{-1} $ZnSO_4 \cdot 7 H_2O$; 20 g l^{-1} $CuSO_4 \cdot 5 H_2O$; 25 g l^{-1} $MnSO_4$; 20 ng ml^{-1} EGF; 0 ng ml^{-1} hepatocyte growth factor; 1 mM l-ascorbic acid 2-phosphate and 90 g ml^{-1} heparin. Both cell types were seeded in the device media channels at a density of 4M cells ml^{-1} and left to attach for 30 min. Unattached cells were then washed out with fresh media and devices were cultured for the indicated time periods in a variable incubator (5% CO_2, 37°C).

S3. Derivation of the variable function for simulation of Hepatocyte culture

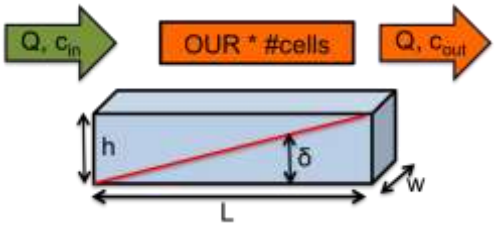
For hepatocyte (HEP) culture in microfluidic devices, the experimental data could initially not be reproduced with simulations using a fixed average oxygen uptake rate (literature value for OUR ~ 500 pmol s^{-1} 10^{-6} cells). Hence, a dynamic behavior of oxygen consumption was postulated for HEP culture in microfluidic devices due to the effects of hepatic hibernation.[4, 5] A polynomial function provided the best fit to the literature data. However, based on our experimental results, we considered a more conservative oxygen consumption rate (OUR=83) at very low oxygen levels compared to the value predicted in literature (OUR=350). Good agreement between experiment and simulation was achieved by assuming a linear relationship between OUR and oxygen partial pressure in the devices. OUR values and the resulting variable functions used in the simulations are summarized below:

P_{O2} [Torr]	O_2 [%]	OUR_{Lit} [4, 6] [pmol s^{-1} 10^{-6} cells]	OUR_{mod} [pmol $s^{-1}10^{-6}$ cells]
100	13.47	700	700
50	6.74	360	360
20	2.70	350	83
0	0	0	0

OUR_{Lit}: $R_c = -((8.1544 * c^3)-(1.6626 * c^2)+(0.1007c)-(3 * 10^{-16}))$

OUR_{mod}: $R_c = -((0.0256 * c)-(10^{-4}))$

S4. Calculation of the minimum flowrate required to counteract oxygen depletion



For a defined volume containing a cell monolayer at $h_c \leq 10$ µm, the flow of oxygen out of the system is defined by the cellular oxygen uptake rate (OUR) and a minimum flow of oxygen downstream (c_{out} is arbitrarily set to 0.1 mol m^{-3}).

$$\dot{m}_{O2,out} = OUR * \#_{cells} + Q * c_{out} \quad (1)$$

The oxygen flow into the system is determined by the flowrate Q of the media and its oxygen concentration c.

$$\dot{m}_{O2,in} = Q * c_{in} \quad (2)$$

where c_{in} = 0.17075 mol m^{-3} is the concentration of oxygen in water at 37° C.[7-9]

The minimum flowrate Q_{min} required to compensate for the losses due to cellular consumption ($\dot{m}_{O2,out} = \dot{m}_{O2,in}$) can be calculated by combining eqs. 1&2.

$$Q_{min} = \frac{OUR*\#_{cells}}{c_{in}-c_{out}} \quad (3)$$

Assuming h_c<<w, the shear stress τ exerted on the cell layer is then estimated from the Hagen-Poiseuille equation:

$$\tau = 4\left(\frac{Q_{min}*\mu}{h_c^2*w}\right) \quad (4)$$

where µ = 0.718 *10^{-3} kg m^{-1} s^{-1} is the dynamic viscosity of water at 37° C.[10]

Average OURs values from literature were used to calculate Q_{min} and the resulting shear stresses τ. The channel dimensions used are h = 150 µm, w = 500 µm and L = 15 mm.

	OUR[5, 11, 12] [mol s^{-1}]	# cells	Q_{min} [m^3 s^{-1}]	τ [Pa]
HEP	$5 * 10^{-16}$	3859	$2.73 * 10^{-11}$	1.57
EC	$2 * 10^{-17}$	11796	$3.33 * 10^{-12}$	0.19

In order to compare the influence of convection and diffusion effects on oxygen mass transport, we calculated the Peclet number for the high flowrate scenario represented by HEP culture in COC.

$$Pe = \frac{u*h}{D} \tag{5}$$

The free stream velocity u was obtained from the flowrate Q_{min} and the cross-section A = h * w of the media channel:

$$u = \frac{Q_{min}}{A} = 3.64 * 10^{-4}\ \frac{m}{s} \tag{6}$$

At a characteristic length of h = 150 µm (channel height) and a diffusivity of oxygen in media of D = 3.35 * 10^{-9} m^2 s^{-1}, this results in a Peclet number of 16.3 for HEP. Peclet numbers >1 indicate that oxygen distribution is no longer diffusion dominated and boundary layer effects have to be taken into account.
First, the entrance length l_e is calculated to make sure that at a given distance into the channel the flow profile is fully developed. The dimensionless entrance length number E_l is correlated with the entrance length l_e and the Reynolds number Re.

$$E_l = \frac{l_e}{h} = 0.06 * Re \tag{7}$$

Re is an indicator of the type of flow in a tube (laminar for Re < 2300, transient or turbulent for Re > 4000). For a rectangular tube, Re is a function of the hydraulic diameter d_h:

$$Re = \frac{u*\varrho}{\mu} * d_h = \frac{u*\varrho}{\square} * \frac{2*h*w}{(h+w)} \tag{8}$$

where ρ = 1000 kg* m^{-3} is the density of water.

A Reynolds number of Re = 0.12 and the resulting entrance length of l_e = 1.05 µm demonstrate that a laminar flow profile is established and fully developed immediately after the channel inlet. The concentration boundary layer thickness δ_c at a distance x from the channel inlet is determined by the effects of momentum and mass diffusion (*via* the Schmidt number Sc):[13]

$$\delta_c = \frac{x}{\sqrt{Re}} * \sqrt[3]{Sc} = \frac{x}{\sqrt{Re}} * \sqrt[3]{\frac{\mu}{\rho*D}} \tag{10}$$

At a distance of x = 100 µm into the channel the boundary layer thickness is in the range of millimeters, and the assumption of uniform oxygen concentration over the cross-section is thus warranted.

S5. Calculation of oxygen levels inside a gas-permeable laminate-sealed channel

The flux of oxygen through a gas permeable membrane of thickness h_m is described by the permeation equation (derived from Fick`s law):

$$J_{in} = \frac{D*S}{h_m}(p_1 - p_2) = \frac{P}{h}(p_1 - p_2) \quad (11)$$

where P, S and D represent the permeability, solubility and diffusivity of oxygen in the material, and p_1 is the partial pressure of oxygen in the atmosphere (21227 Pa).

The rate of oxygen consumption per unit area inside the system is calculated from OUR*#cells.

$$J_{out} = \frac{OUR*\#_{cells}}{w*L} \quad (12)$$

Assuming steady-state conditions ($J_{in} = J_{out}$), the total oxygen flux into the device is balanced by the rate of oxygen consumption by the cells, giving rise to the expression:

$$\Delta p = p_1 - p_2 = J_{out} * \frac{h_m}{P} \quad (13)$$

The resulting partial pressures in the devices for EC and HEP culture at a given membrane thickness h and material are summarized below. The permeability values used for PDMS and PMP are 800 Barrer and 22.3 Barrer, respectively.[14, 15]

	PMP			PDMS		
h_m [µm]	50	100	500	50	100	500
$p_{O2,Hep}$ [Pa]	19505	17782	3998	21179	21132	20747
$p_{O2,EC}$ [Pa]	21017	20806	19121	21222	21216	21169

As shown, normoxic partial pressure levels (> 5000 Pa)[5, 16] can be maintained in all devices except for HEP on very thick PMP laminates. However, in an experimental setup additional oxygen may be supplied from diffusion within the device or by establishing moderate media flowrates as indicated above.

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