MODEL ANALYSIS OF OXYGEN DIFFUSION/CONSUMPTION FOR CELL CULTURE SYSTEM TO OPTIMALLY DESIGN SCAFFOLD AND MICROBIOCHIP

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Introduction

Recently, devices utilizing biological cells such as hybrid artificial organ and cell chip receive much attention in the biomedical field. We need to optimally design such a device to cultivate the specific cells and maintain their function in it. At first, we have assumed that the dominant factor to cultivate cells is the oxygen supply to the cells and presented a mathematical model to simulate the cell growth on a scaffold. In the model, we have also assumed that the size of an organoid, which is a micro tissue formed by cells, is limited by the oxygen supply to the cultivated cells, and oxygen transport occurs solely by diffusion in the organoids cultivated on a scaffold. This situation imposes severe restrictions on the system; necrosis due to oxygen deficiency will occur in regions where the partial oxygen pressure is lower than a certain critical value. The maximum sizes of cylindroids (cylindrical organoids) estimated by the model coincide well with the experimental data presented in the previous literatures, showing the validity of the model (1).

In this study, based on the model with the assumptions, we make some concrete proposals to optimally design a microbiochip, where cells are cultivated and handled.

Formulation of Models

In this study, we discuss glucose transfer besides of oxygen transfer in a microchip, where cells are cultivated. The basic design of the chamber is shown in Figure 1. The cells are cultivated in the cell culture chamber and forms hemispheroids (hemispheric organoids).

In this microchip, the channel to supply oxygen and nutrition to the chamber is equipped on the cell culture chamber isolated by a semi-permeable membrane. In a steady state, the flux of oxygen or glucose transferred from the channel to the chamber, J [$gm^{-2}s^{-1}$], can be expressed as

$$J = m_c t_c \qquad (1)$$

where m_c [$gm^{-3}s^{-1}$] and t_c [m] denote the consumption rate of oxygen or glucose coming from the metabolism of the cultivated cells and the apparent thickness of the cultivated cells, which is calculated as $2\pi r^3/3l^2$, respectively.

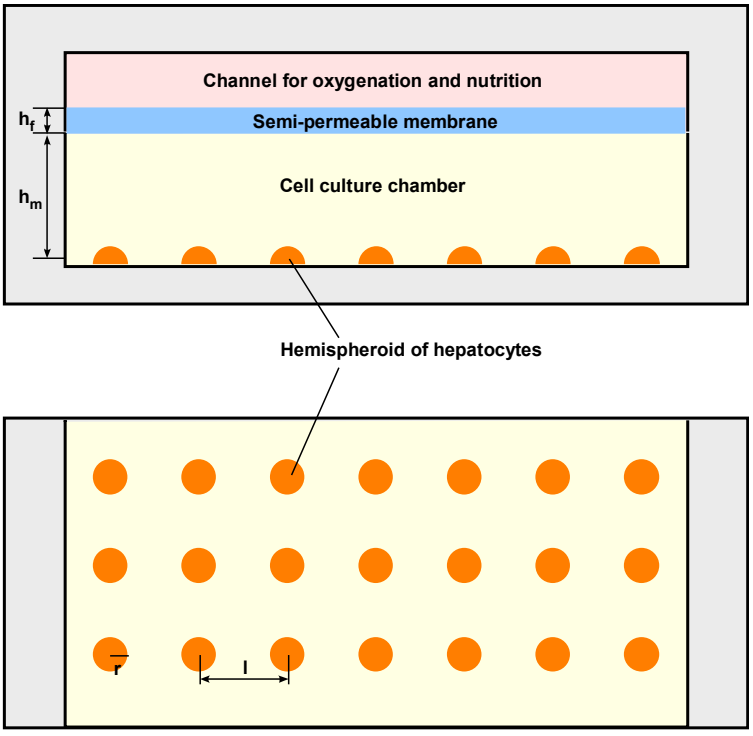

Figure 1. Basic design of cell-array chip.

Here, the thickness and diffusivity of the membrane are h_f [m] and D_f [m^2s^{-1}], respectively, and the thickness and diffusivity of the medium filled in the cell culture chamber are h_m [m] and D_m [m^2s^{-1}], respectively. Then, in a steady state, the following equation can be formulated as

$$J = \left(C_o - C_c\right) \Big/ \left(\frac{h_m}{D_m} + \frac{h_f}{D_f} \right) \qquad (2)$$

where C_o [gm^{-3}] and C_c [gm^{-3}] denote the concentrations of oxygen or glucose at the channel-side surface of the membrane and the interface between the medium and the apparent cell layer, respectively.

Combining eq. (1) with eq. (2) and substituting t_c by $2\pi r^3/3l^2$, the following equation can be obtained,

$$C_c = C_o - \frac{2\pi r^3 m_c h_{eq}}{3 D_m l^2} \qquad (3)$$

where h_{eq} [m] is the equivalent thickness of the diffusive layer composed of only the medium, and is for glucose as

$$h_{eq} = h_m + \frac{D_m h_f}{D_f}. \qquad (4)$$

For oxygen, we can discuss in terms of the partial pressure, p [mmHg] using the solubility of oxygen α [$gm^{-3}mmHg^{-1}$] as,

$$p_c = p_o - \frac{2\pi r^3 m_c h_{eq}}{3 \alpha_m D_m l^2} \qquad (5)$$

$$h_{eq} = h_m + \frac{\alpha_m D_m h_f}{\alpha_f D_f} \qquad (6)$$

where α_m [$gm^{-3}mmHg^{-1}$] and α_f [$gm^{-3}mmHg^{-1}$] are the solubilities of oxygen in the medium and membrane, respectively.

Next, we study a cell-array chip, where glucose is supplied from the feeding chambers, which are equipped at the both ends of the cell culture chamber. In this case, the concentrations of oxygen and glucose are assumed to be constant and the concentration profiles are calculated using the one-dimensional Fick's second law. The mass balance gives the following equation,

$$\frac{d^2 C}{dx^2} = \frac{m_c}{D_f} \qquad (7)$$

where x [m] is the position along the cell culture chamber, which is zero at the center of the chamber. Eq. (7) can be integrated with the boundary conditions; $x = \pm L/2$: $C = C_s$, where L [m] is the length of the cell culture chamber and f is the ratio of the cross-section area of the cultivated cells to that of the cell culture chamber, and then, the following equation is obtained,

$$C = \frac{m_c f}{8}\left(4x^2 - L^2\right) + C_s. \qquad (8)$$

Results and Discussion

First of all, we estimated the oxygen or glucose supply required to cultivate cells in a cell culture chamber. Roughly and generally, the next equation can be formed in a steady state for a cell culture system,

$$J = D\frac{\Delta C}{\Delta x} = M_c \qquad (9)$$

where M_c [$gm^{-2}s^{-1}$] denotes the consumption rate of oxygen or glucose of the cultivated cells per unit area. This equation means that oxygen or glucose with the diffusion coefficient, D [m^2s^{-1}], is transferred by the concentration slop, $\Delta C/\Delta x$, at the flux, J, and J is balanced with the consumption rate of the substance by the cells. So, we can roughly estimate the allowance in $\Delta C/\Delta x$ for a cell chip by M_c/D. Because 6 mol oxygen is needed for the oxidization of 1 mol glucose on the basis of stoichiometry and the ratio of the molecule weight of oxygen to that of glucose is 1:5.6, M_c is estimated to be almost the same for oxygen and glucose. The value of D for oxygen in water at 37°C is $2.1\text{x}10^{-9}$ m^2s^{-1} (1) and that for glucose is estimated as $9.3\text{x}10^{-10}$ m^2s^{-1} (2).

We think we should develop a cell chip, which can be used in an ordinary incubator with 5% carbon dioxide at 760 mmHg, to utilize it widely. Therefore, the maximum value of ΔC for oxygen is estimated as 29 g m^{-3} (the partial pressure of oxygen in the incubator: 675 mmHg, α in water at 37°C: $4.32\text{x}10^{-2}$ $gm^{-3}mmHg^{-1}$(1)). On the other hand, the maximum glucose concentration of an ordinary medium is $4\text{x}10^3$ g m^{-3}, which is considered to be the maximum value of ΔC for glucose. Using these estimated values, Δx can be calculated as $6.1\text{x}10^{-8}/M_c$ for oxygen and $3.8\text{x}10^{-6}/M_c$ for glucose. This estimation indicates that we can design a cell-array chip paying attention only to oxygen supply, if oxygen and glucose are supplied through the same pathway.

Figure 2 shows the oxygen partial pressure at the interface between the medium and the apparent cell layer, p_c [mmHg], which is calculated against the lattice constant, l [m] (see Figure 1), using eq. (3) on the assumption that the radius, r [m], of the cultivated hemispheroids (see Figure 1) is 25 μm, α is $4.32\text{x}10^{-2}$ $gm^{-3}mmHg^{-1}$, p_o [mmHg], which is corresponding to c_o in eq. (3), is 148 mmHg (assuming that the cell-array chip is handled in the atmosphere) and h_{eq} is 2 mm. We have to discuss about the magnitude of m_c, which is various in previous literatures even for the same cell type, but we have presented that m_c for hepatocytes, which are the most promising for a cell-array chip, is likely much larger than those of the other cell types, which are estimated as 1 $gm^{-3}s^{-1}$ (1).

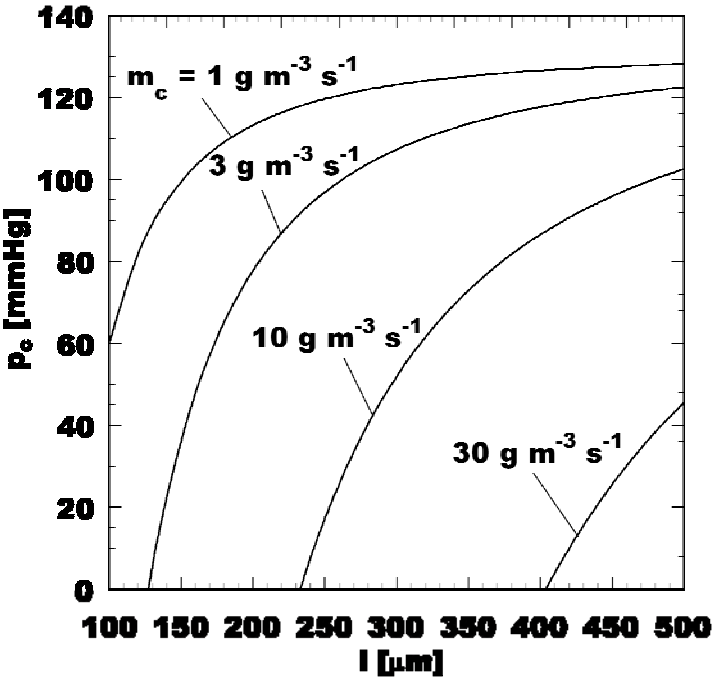

Figure 2. Partial pressure profile of oxygen in a cell-array chip.

As can be seen in Figure 2, it is not so easy to optimally design a cell-array chip, if we want to cultivate the hemispheroids closely, because we have presented that the cells never grow at p_c under 40 mmHg. Here, we assume h_{eq} to be 2 mm as a realistic value. It is well known that the diffusivity of a solute in a hydrogel, D_G, can be estimated as $D_G = HD_W$, where H is the water content [-] and D_W [m^2s^{-1}] is the diffusivity of the solute in water (3). Here, it is reasonable to assume that $\alpha_m = \alpha_f = \alpha_w$, where α_w is the solubility of oxygen in water, and $D_G = D_W$ for oxygen, because the medium is usually a dilute solution and a hydrogel with a

higher H is used. Therefore, the following relation is obtainable from eq. (4) in the case of Figure 2.

$$h_m + h_f / H = 2\times10^{-3} \qquad (10)$$

It is not so difficult to form a hydrogel membrane fulfilling eq. (10), referring to the commercially available hemodialysis membranes (4). For example, a microchip with 1 mm of h_m equipped with a semi-permeable membrane with 500 μm of h_m and 0.5 of H is realistic.

We can design another setup for oxygen and glucose supply in a cell-array chip, where oxygen is supplied through the upper wall of the cell culture chamber and glucose is supplied by diffusion from the feeding chambers, which are equipped at the both ends of the cell culture chamber. In this case, it can be easily confirmed for oxygen to be supplied enough. For example, D_f and α_f of poly(dimethylsiloxane), which is the most universal material to prepare a microchip, are roughly estimated as $3.4\text{x}10^{-3}$ m^2s^{-1} and $3.1\text{x}10^{-1}$ $gm^{-3}mmHg^{-1}$ (5). Therefore, h_{eq} is obtainable using eq. (6) as,

$$h_{eq} = h_m + 0.30h_f \qquad (11)$$

and $h_{eq} \approx h_m$, because the second term in the right hand of eq. (11) is practically negligible.

The concentration profiles of glucose along a cell culture chamber with L = 20 mm and f = 0.01 were calculated using eq. (8) at C_s = 4 g/L as shown in Figure 3. For example, f = 0.01 is obtainable for a cell culture chamber with the height of 200 μm, where cells are cultivated in a layer of the thickness of 2 μm, or one with the height of 220 μm, where hemispheroids of the radius 25 μm are cultivated in the square lattice arrangement of l = 120 μm. This result indicates that it is pretty difficult to design a large-size cell-array chip, where glucose is supplied in the above-mentioned way, if the glucose consumption rate of the cells cultivated in the chip is high as suggested in ref. (1). In that case, the setup shown in Figure 1 should be used.

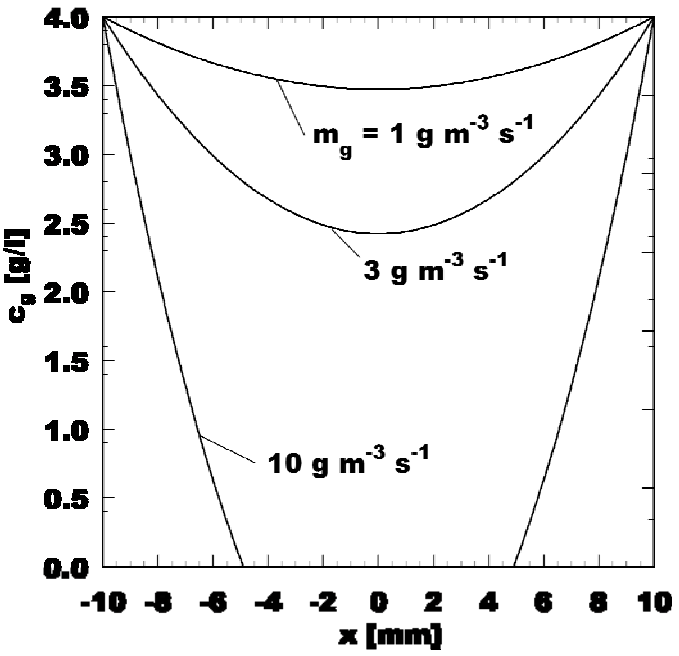

Figure 3. Concentration profile of glucose in a cell-array chip.

Conclusions

Oxygen supply was estimated to impose much more critical condition on the design of cell-array chip than glucose supply. Two types of the setup to supply oxygen and glucose to the cells cultivated in the cell-array chip have been discussed. The optimal design shows that a setup to supply oxygen and glucose from the upper wall of the cell culture chamber are required, if the consumption rates of the cultivated cells for oxygen and glucose are higher than 10 $gm^{-3}s^{-1}$.

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