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A Steady-State Mass Transfer Model of Removing CPAs from Cryopreserved Blood with Hollow Fiber Modules

Weiping Ding¹, Xiaoming Zhou¹, Shelly Heimfeld², Jo-Anna Reems³, and Dayong Gao^{1,*}

¹Department of Mechanical Engineering, University of Washington, Seattle, WA 98195, USA

²Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA

³Puget Sound Blood Center, Seattle, WA 98104, USA

Abstract

Hollow fiber modules are commonly used to conveniently and efficiently remove cryoprotective agents (CPAs) from cryopreserved cell suspensions. In this paper, a steady-state model coupling mass transfers across cell and hollow fiber membranes is theoretically developed to evaluate the removal of CPAs from cryopreserved blood using hollow fiber modules. This steady-state model complements the unsteady-state model which was presented in our previous study. As the steady-state model, unlike the unsteady-state model, can be used to evaluate the effect of ultrafiltration flow rates on the clearance of CPAs. The steady-state model is validated by experimental results and then is compared with the unsteady-state model. Using the steady-state model, the effects of ultrafiltration flow rates, NaCl concentrations in dialysate, blood flow rates and dialysate flow rates on CPA concentration variation and cell volume response are investigated in detail. According to the simulative results, the osmotic damage of red blood cells (RBCs) can easily be reduced by increasing ultrafiltration flow rates, increasing NaCl concentrations in dialysate, increasing blood flow rates or decreasing dialysate flow rates.

Keywords

Mass Transfer; Cryoprotective Agent; Ultrafiltration; Cell Volume; Hollow Fiber

1. Introduction

Cryopreservation processes of biological materials such as cells, tissues and organs, generally consist of five important steps: addition of CPAs, freezing, storage under low temperature, thawing and removal of CPAs [1,2]. Each of these steps needs to be properly dealt with to ensure successful cryopreservation. In particular, since the addition and removal processes of CPAs may result in serious osmotic damage of cells and result in the loss of the tissue [1-4], the focus of this paper is on the removal process of CPAs.

Traditionally, one-step and multi-step methods are used to remove CPAs from cryopreserved cells [1-10]. Compared to the one-step method, the multi-step method can alleviate osmosis damage of cells, but it is more complex to operate, more time consuming and more costly. One-step and multi-step methods have some common disadvantages. One is that the centrifugal effect can easily cause cell clumping and cell loss [11,12]. Another is that neither of these two

*Corresponding author, dayong@u.washington.edu.

methods is suitable for a large cell suspension, such as is encountered when removing CPAs from cryopreserved blood for clinical transplant.

Currently, hollow fiber modules offer an alternative approach to remove CPAs [13,14]. Common hollow fiber modules consist of thousands of hair-like hollow fibers which are potted at two ends and inserted into a cylindrical vessel. Hollow fiber modules can be used not only to separate solutes from solutions but also to condense solutions. During the removal of CPAs from cryopreserved blood, the cryopreserved blood/cell suspension with the CPAs flows inside of fibers and the dialysate/washing solution counter-current passes outside of the fibers. Due to pressure and concentration differences across cell and fiber membranes, CPAs in cells are transported out of the cells and then removed. If the CPA concentration in the cryopreserved blood does not reach the desired value, for example 5% of the initial CPA concentration, the removal process is repeated (Fig.1). Use of hollow fiber modules can effectively reduce cell osmotic damage and avoid cell clumping. Another advantage is that this method can conveniently and efficiently handle large cryopreserved cell suspensions. The basic idea of this method is that by running cryopreserved blood in a hollow fiber module, the successively decreasing CPA concentration outside of cells which is similar to the stepwise decreasing CPA concentration in the multi-step method is indirectly realized.

In previous work, we used an unsteady-state model to simulate the above process theoretically [13]. Although the unsteady-state model can trace real-time variations of cell volume and CPA concentration during the whole process, it is based on the assumption that the effect of the local ultrafiltration across the fiber membrane can be assumed negligible. Thus, the unsteady-state model cannot be used to study the effect of ultrafiltration flow rates (Unless otherwise specified, ultrafiltration denotes total ultrafiltration in this paper) on the CPA removal. However, in practice, increasing ultrafiltration flow rates is often used not only to enhance mass transfer efficiency but also to condense cell suspensions. In addition, in order to improve the calculation accuracy of the unsteady-state model one has to adopt very tiny time step-sizes and divide the limited blood volume into small enough units. This consumes a lot of computer resources and is not convenient to study the effects of various parameters on mass transfer. So a versatile model is needed for practical application.

In this paper, a steady-state model for studying the removal of CPAs from cryopreserved blood with hollow fiber modules is deduced. This model can be used to investigate the effects of different operation conditions on mass transfer. Although steady-state is an ideal operation condition for a limited blood volume, in practice it can still be reached approximately when the ratio of blood volume to fiber volume is very large. Moreover, for a given blood volume, one could also use a mini-hollow fiber module to approximate the ideal condition.

2. Theory

The steady-state model in this paper was developed under the following assumptions[15-20]: (a) The inlet and outlet configurations of hollow fiber modules are ideal and can produce even flow fields on both sides of fibers; (b) Fibers are identical, rigid, and aligned axially i.e. the flow distribution is consistent along the hollow fiber length; (c) Flows are isothermal and laminar; (d) The effect of diffusion on mass transfer is negligible; (e) The effect of the module wall on shell hydraulics is negligible; (f) Hydrostatic forces are negligible; (g) Kinetics of solute adsorption is negligible; (h) The expansion of fibers under wet conditions is ignored.

2.1 Transports across hollow fiber membrane

In this study, we assumed that all solutions only contain two solutes: glycerol and sodium chloride (NaCl). Glycerol, a CPA, is semi-permeable to human RBC membrane and NaCl is considered to be non-permeable to human RBC membrane. However, both glycerol and NaCl

are supposed to be permeable to the fiber membrane because of big pores on the surface of the fiber membrane.

Based on the literature [21], the transmembrane volume and solute fluxes can be described as

$$J_{v,h} = L_{p,h}(P_d - P_b) \quad (1)$$

$$J_{s,h} = \bar{m}_{s,h} J_{v,h} + P_{s,h}(m_{s,3} - m_{s,2}) \quad (2)$$

$$J_{n,h} = \bar{m}_{n,h} J_{v,h} + P_{n,h}(m_{n,3} - m_{n,2}) \quad (3)$$

where subscripts 2 and 3 refer to the extracellular part (outside of cells but inside of fibers) and the dialysate side part (outside of fibers) respectively, P_b and P_d represent pressures on blood and dialysate sides respectively, subscripts s and n represent glycerol and NaCl respectively, subscript h denotes hollow fiber, J_v , J_s and J_n are volume flux, glycerol flux and NaCl flux across unit membrane area respectively, L_p , P_s and P_n are hydraulic permeability, CPA permeability, NaCl permeability respectively (P_s and P_n also are overall mass transfer coefficients across the fiber membrane here [15,19]), m_s is CPA molality, m_n is NaCl osmolality, $\bar{m}$ is the logarithmic mean concentration in the membrane.

2.2 Axial pressure drops and flow rates

Pressure distributions along the axial direction on both sides of the hollow fibers can be calculated by Hagen–Poiseuille and modified Hagen–Poiseuille equations respectively [15, 22]:

$$\frac{dP_b}{dx} = - \frac{8\mu_b}{N\pi r_i^4} Q_b \quad (4)$$

$$\frac{dP_d}{dx} = - \frac{8\mu_d(r_M + Nr_0)^2}{\pi(r_M^2 - Nr_0^2)^3} Q_d \quad (5)$$

where subscripts b and d represent blood and dialysate sides respectively, r_M , r_i and r_o are module inner radius, fiber inner radius and fiber outer radius respectively, Q is the flow rate, N is the number of fibers, and μ is viscosity (Fig.2 shows the schematic of control volume; the gray region is the membrane whose thickness is assumed to be negligible).

The flow rates on both sides of the fibers can be calculated by continuity equations:

$$\frac{dQ_b}{dx} = 2N\pi r_i J_{v,h} \quad (6)$$

$$\frac{dQ_d}{dx} = 2N\pi r_i J_{v,h} \quad (7)$$

2.3 Variations of cell volume, CPA and NaCl concentrations inside of cells

Assuming that the hydrostatic pressure difference across the cell membrane is zero, variations of cell volume and intracellular glycerol concentration can be presented as follows [4-6]:

$$\frac{dV_c}{dt} = L_{p,c} A_c R T [(m_{n,1} - m_{n,2}) + \sigma_c (m_{s,1} - m_{s,2})] \quad (8)$$

$$\frac{dm_{s,1}}{dt} = \frac{(1 + \bar{V}_s m_{s,1})^2}{V_c - V_{bc}} \left\{ [\bar{m}_{s,c} (1 - \sigma_c) - \frac{m_{s,1}}{1 + \bar{V}_s m_{s,1}}] \frac{dV_c}{dt} + P_{s,c} A_c (m_{s,2} - m_{s,1}) \right\} \quad (9)$$

The above two equations were originally used to study the variations of cell volume and intracellular CPA concentration for the traditional CPA removal methods. In this study, what we expect is that cell volume and intracellular glycerol concentration change as functions of position under the steady-state condition. For one cell, when it moves from the inlet to the outlet of the hollow fiber module, cell position, cell volume and intracellular glycerol concentration are functions of time (Lagrangian reference frame); however, for a given location at any time, cell volume and intracellular glycerol concentration at this location keep constant under the steady-state situation (Eulerian reference frame). Due to the facts that the position of each cell is related to its moving time and that the behaviors of all cells are identical under the steady-state condition, the changes of any cell with respect to time, or position along the flow direction, are equivalent to the steady-state space distributions of the above variations.

Considering the relations $\frac{dV_c}{dt} = \frac{Q_b}{S_f} \frac{dV_c}{dx}$ and $\frac{dm_{s,1}}{dt} = \frac{Q_b}{S_f} \frac{dm_{s,1}}{dx}$ (S_f is equal to $N\pi r_i^2$), the above two equations can be rewritten as

$$\frac{dV_c}{dx} = - \frac{L_{p,c} R T A_c S_f}{Q_b} [(m_{n,2} - m_{n,1}) + \sigma_c (m_{s,2} - m_{s,1})] \quad (10)$$

$$\frac{dm_{s,1}}{dx} = \frac{(1 + \bar{V}_s m_{s,1})^2}{V_c - V_{bc}} \left\{ [\bar{m}_{s,c} (1 - \sigma_c) - \frac{m_{s,1}}{1 + \bar{V}_s m_{s,1}}] \frac{dV_c}{dx} + \frac{P_{s,c} A_c S_f}{Q_b} (m_{s,2} - m_{s,1}) \right\} \quad (11)$$

where subscript c denotes cell, subscript 1 refers to the intracellular part (inside of cells), A is the cell membrane area, which is assumed to be constant, V is the volume, R is the universal gas constant, T is the absolute temperature, S_f is the total cross-section area of fibers, $\bar{V}_s$ is the CPA partial molar volume, σ_c is the reflection coefficient of glycerol to the cell membrane, and $V_{bc} = V_{iso} V_{bp}$ (V_{bc} is the osmotically inactive cell volume, V_{iso} is the isotonic volume of cells, and V_{bp} is the percentage of osmotically inactive cell volume at the isotonic condition).

For the impermeable solute NaCl inside of cells, its total molar number keeps constant. Then NaCl osmolality can be calculated by:

$$m_{n,1}=m_{n,1}(0)\frac{V_c(0)-V_{bc}-V_{s,1}(0)}{V_c-V_{bc}-V_{s,1}} \quad (12)$$

where superscript 0 represents values at $x=0$, and V_S is the CPA volume.

2.4 Variations of CPA and NaCl concentrations outside of cells but inside of fibers

According to the concentration conservation of CPAs, the following equation can be obtained

$$\frac{d(Q_{w,b}m_{s,2}+\beta n_{s,1})}{dx}=2N\pi r_i J_{s,h} \quad (13)$$

where subscript w refers to water, n is the molar number, and β is related to cell density γ and defined as the number of cells per unit time ($\beta=\gamma Q_{bin}$).

The volume of cell suspension with CPA mainly includes three parts: water, CPA and cell volumes:

$$Q_b=Q_{w,b}+Q_{s,b}+\beta V_c \quad (14)$$

Considering $Q_{s,b}=m_{s,2}Q_{w,b}$, V_s , equation (14) is changed to be

$$Q_{w,b}=(Q_b-\beta V_c)/(1+\bar{V}_s m_{s,2}) \quad (15)$$

Combining equations (13) and (15), the following equation is acquired

$$\frac{dm_{s,2}}{dx}=\frac{(1+\bar{V}_s m_{s,2})^2}{Q_b-\beta V_c}\left[2N\pi r_i(J_{s,h}-\frac{m_{s,2}}{1+\bar{V}_s m_{s,2}}J_{v,h})+\beta(\frac{m_{s,2}}{1+\bar{V}_s m_{s,2}}-\frac{m_{s,1}}{1+\bar{V}_s m_{s,1}})\frac{dV_c}{d}-\beta\frac{V_c-V_{bc}}{(1+\bar{V}_s m_{s,1})^2}\frac{dm_{s,1}}{d}\right] \quad (16)$$

Because NaCl is impermeable to the cell membrane, the concentration conservation equation is

$$\frac{d(Q_{w,b}m_{n,2})}{dx}=2N\pi r_i J_{n,h} \quad (17)$$

Combining equations (15) and (17), one can get

$$\frac{dm_{n,2}}{dx}=\frac{1+\bar{V}_s m_{s,2}}{Q_b-\beta V_c}\left[2N\pi r_i(J_{n,h}-\frac{m_{n,2}}{1+\bar{V}_s m_{s,2}}J_{v,h})+\beta\frac{m_{n,2}}{1+\bar{V}_s m_{s,2}}\frac{dV_c}{dx}+\frac{m_{n,2}(Q_b-\beta V_c)\bar{V}_s}{(1+\bar{V}_s m_{s,2})^2}\frac{dm_{s,2}}{dx}\right] \quad (18)$$

2.5 Variations of CPA and NaCl concentrations outside of fibers

Using the same steps as in section 2.4, the equation for simulating the variation of CPA concentration outside of fibers along blood flow direction is

$$\frac{d(Q_{w,d}m_{s,3})}{dx} = 2N\pi r_i J_{s,h} \quad (19)$$

With $Q_{w,d} = Q_d / (1 + \bar{V}_s m_{s,3})$, the equation (19) can be rewritten as

$$\frac{dm_{s,3}}{dx} = \frac{(1 + \bar{V}_s m_{s,3})^2}{Q_d} \{ 2N\pi r_i [J_{s,h} - \frac{m_{s,3}}{1 + \bar{V}_s m_{s,3}} J_{v,h}] \} \quad (20)$$

For the NaCl concentration outside of fibers, one could consider both sides of hollow fibers to be a big control volume and then the following equation is acquired (“-” denotes that blood and dialysate flow counter-currently),

$$\frac{d}{dx} (Q_{w,b}m_{n,2} - Q_{w,d}m_{n,3}) = 0 \quad (21)$$

Then,

$$\frac{dm_{n,3}}{dx} = \frac{1 + \bar{V}_s m_{s,3}}{Q_d} \left[2N\pi r_i (J_{n,h} - \frac{m_{n,3}}{1 + \bar{V}_s m_{s,3}} J_{v,h}) + \frac{m_{n,3} Q_d \bar{V}_s}{(1 + \bar{V}_s m_{s,3})^2} \frac{dm_{s,3}}{dx} \right] \quad (22)$$

In the hollow fiber module, the glycerol outside of cells (on the blood side) is shipped to the dialysate side due to the pressure and concentration differences across the fiber membrane and thereby the glycerol outside of cells decreases continuously. The decrease of the glycerol outside of cells leads to the concentration differences across the cell membrane. Because of these concentration differences the glycerol inside of cells is finally removed. The change of cell volume depends on the volume flux which is caused by both glycerol and NaCl concentration differences across the cell membrane (See equation 8 or 10). If the volume flux is positive, the volume of cells will increase; otherwise the volume will decrease. The cell volume usually increases at first and then decreases; however, the CPA concentration inside of cells always decreases. The mass transfer does not stop until the volume and solute fluxes reach new equilibrium simultaneously [13].

3. Simulation Procedures

3.1 Boundary Conditions

Boundary conditions used in this study are listed in Table 1. The total ultrafiltration flow rate Q_{uf} is only a boundary condition. According to $Q_{uf} = Q_{bout} - Q_{bin}$, for the given Q_{bin} and Q_{uf} , $Q_{bout} = Q_{bin} + Q_{uf}$. So in this paper, boundary conditions for flow rates are Q_{bin} , Q_{din} and Q_{uf} . In addition, the boundary values of V_{co} , $m_{s,1}$, and $m_{n,1}$ at $x = 0$ could be thought to be the initial conditions for one cell. V_{co} can be calculated according to the boundary values of $m_{s,1}$, $m_{n,1}$ and V_{iso} .

3.2 Parameter setting

One experimental setup presented in the literature [23] was established to determination hydraulic permeability of the fiber membrane. In the experiments, two pumps (MasterFlex[®], Model No.: 77521-40; Easy-load[®] II, Model No.: 77200-62; MasterFlex[®] Tubing L/STM 15; Cole-Parmer Co., USA) and one hollow fiber module (Polyflux[™] 6LR, Gambro Co., Sweden)

were used. Osmolality/concentration was measured by VAPRO® Vapor Pressure Osmometer (Wescor, Inc., USA). The total ultrafiltration flow rates were determined by a balance (Scout™ Pro, Model No.SP4001, Ohaus Corporation, USA) according to the change of solution weight.

Two groups of experiments were performed. In the first group, the flow rate of dialysate was set to be 199.28ml/min and four blood flow rates 220.69, 256.06, 291.43, and 326.8ml/min were conducted. In the second group, the flow rate of dialysate was 553.03ml/min, and five blood flow rates were 220.69, 244.27, 267.85, 291.43 and 315.01ml/min. Hydraulic permeabilities measured under these two conditions were 1.69×10^{-11} and 1.92×10^{-11} m/s/Pa respectively. In this study, the average hydraulic permeability 1.805×10^{-11} m/s/Pa was used.

Glycerol and NaCl permeabilities of Polyflux™ 6LR were acquired by fitting experimental data under steady-state conditions. In the experiments, the standard flow conditions were the blood flow rate at the blood inlet $Q_{bin} = 252.575$ ml/min, the dialysate flow rate at the dialysate inlet $Q_{din} = 536.325$ ml/min, and the ultrafiltration flow rate $Q_{uf} = 27.175$ ml/min. After steady-state was reached, five samples at the blood outlet were taken to fit diffusive permeability. The solution containing glycerol or NaCl flows inside of hollow fibers, and pure water as dialysate flows outside of hollow fibers (Fig.1). The inlet concentrations of glycerol and NaCl were 1084mOsm and 561mOsm respectively. The fitted diffusive permeabilities of glycerol and NaCl were 2.398×10^{-2} cm/min and 3.785×10^{-2} cm/min respectively.

In this paper, we assumed that the hydraulic and diffusive permeabilities are constant and not affected by the local pressure and concentration. Properties of the two solutions adopted water properties at 298K. The intracellular solution was supposed to contain NaCl and glycerol only, and V_{bp} was set to be 0.283[24]. Other important parameters from the literature [25,26] are listed in Table 2.

3.3 Numerical Algorithm

Multi-variable Runge-Kutta Algorithm was used to calculate the above equations in this paper. Because of countercurrent flow operation, it is impossible to solve all differential equations by marching from one end of the module to the other. So, a trial-and-error technique, known as “shooting technique”, was used[15]. The equations (4-7) were first calculated, and then other equations were solved. During the calculation of equations (4-7), P_{dout} was set to be zero and a tentative value was assigned to $P_b(0)$. After that, these four equations were integrated by marching from $x=0$ to $x=L$, and the value of $Q_b(L)$ was acquired. For the given Q_{bin} and Q_{uf} , $Q_{bout} = Q_{bin} + Q_{uf}$. If $Q_b(L)$ was not equal to Q_{bout} , the “shooting” process was repeated. During the calculation of other equations, two tentative values were assigned to $m_{n,3}(0)$ and $m_{s,3}(0)$ respectively, $m_{n,3}(L) = m_{d,n,in}$ and $m_{s,3}(L) = 0$ should be shot simultaneously.

4. Results and Discussions

4.1 Validation of steady-state model

The osmolality sum of NaCl and glycerol at both blood and dialysate outlets was used to validate the steady-state model in this paper. The experiments were conducted under various operation conditions: different NaCl concentrations at dialysate inlet, different ultrafiltration flow rates, different blood flow rates and different dialysate flow rates. The theoretical results agree well with the experimental data (Table 3). It is necessary to point out that in the above experiments no RBCs were used, i.e. the model validation was based on the operation condition of zero RBC density. The reason is that once RBCs are added into experiments it is hard to measure the CPA concentrations at the outlet accurately because the mass transfer across the RBC membrane still remains when samples are taken. Although one could measure the steady-state concentrations outside of RBCs, one would have to wait a long time for the equilibrium

to be reached. When the density of RBCs is assumed to be zero, the steady-state model in this study is equivalent to that in the literature [15,19].

4.2 Comparison between steady-state and unsteady-state models

The unsteady-state model is used for the operation conditions with zero or very small ultrafiltration and small volumes of RBC suspensions; whereas the steady-state model could be used under the operation conditions with large ultrafiltration and large volumes of RBC suspensions. The comparison between the two models is to show that both models are consistent when the total ultrafiltration flow rate is zero and the volume of cell suspensions is large enough. During the unsteady-state process, the maximal cell volume in the former blood units entering hollow fibers much earlier is bigger than that in the latter blood units [13]. The former blood units meet the lower glycerol concentration outside of fibers, which leads to the faster glycerol mass transfer from the inside to outside of the fiber membrane and then the lower glycerol concentration outside of cells. So the increase of cell volumes is faster and the maximal cell volume is bigger. As blood enters continuously, the maximal cell volume in hollow fibers finally runs to the value in the steady-state process (Fig.3a). At the same time, the intracellular and extracellular glycerol concentrations also run to those values under the steady-state condition respectively (Fig.3b). Under the simulative condition in this study, the mass transfer of blood units after No. 400 in the unsteady-state process is the same as the one in the steady-state situation. These two models are complementary and in essence are the same. However, there are still some small differences between the theoretical values calculated by these two models. From Fig.3b, the asymptotic values of glycerol concentration at the blood outlet on both sides of the cell membrane from the unsteady-state model are all a little higher than the values calculated by the steady-state model. This phenomena/reason is related to the assumption that blood and dialysate velocities are constant in the unsteady-state model. In practice, because of local ultrafiltration, blood and dialysate velocities all decrease first and then increase when total ultrafiltration is zero. Both actual velocities are lower than the assumed ones. That is to say, the actual mass transfer time of RBCs in each unit is longer than the given value in the unsteady-state model. The shorter time step leads to the deficient calculation and then the departure between the two models. Under the lower local ultrafiltration, the excursion is not obvious, but under the higher local ultrafiltration, the excursion will be magnified (In Fig.3, β was $3.76 \times 10^9/s$ and the initial hematocrit was 10.33%).

4.3 Effects of ultrafiltration flow rates on mass transfer

Ultrafiltration is an important factor affecting the CPA removal efficiency. The change of ultrafiltration flow rates almost does not affect the variations of CPA concentration on both sides of the RBC membrane along blood flow direction, but obviously affects RBC volume change (Fig.4). Although the increase of ultrafiltration flow rates accelerates CPA transport across the hollow fiber membrane, water transport from the inside to outside of the hollow fibers is also enhanced and then compensates the decrease of CPA concentration inside of fibers. The increase of ultrafiltration flow rates causes only a very slight change of CPA concentrations at the blood outlet, but it actually enhances the removal of CPA molar quantity; therefore, the total CPA molar quantity in blood decreases, i.e. the CPA clearance increases. Due to the increase of water flux across the hollow fiber membrane, the water inside of the hollow fibers decreases and thereby the water volume entering the RBCs decreases, relatively. So, the normalized RBC volume with ultrafiltration is less than that without ultrafiltration. In practice, whether the maximal cell volume is small enough to avoid osmotic damage of cells and whether the CPA clearance is high enough to satisfy extensive applications are two important criterions to weigh a CPA washing method. Hence, the increase of ultrafiltration flow rates is a good choice for CPA removal with hollow fiber modules (In Fig.4, β was $7.5 \times 10^9/s$ and the initial hematocrit was 15.50%).

4.4 Effects of NaCl concentrations in dialysate on mass transfer

The usage of hypertonic solutions is another choice to reduce the osmotic damage of RBCs [27]. Hypertonic solutions contain solutes, such as NaCl, not permeable to RBC membranes, which can decrease the osmotic pressure difference between the inside and outside of RBCs and then reduce the transport speed of water from the inside to outside of RBCs. The variation of cell volume is dominated by water transport, so hypertonic solutions can decrease RBC volume. However, hypertonic solutions also could introduce a negative effect. Because of the hypertonic solute concentration, the final cell volume would be less than the shrinkage limit cells can stand (RBC shrinkage limit is about half of its isotonic volume). Accordingly, the shrinkage of cell volume also should be monitored if hypertonic solutions are used as dialysate so that the hypertonic damage of RBCs can be avoided. Fig.5 shows the effects of hypertonic NaCl dialysates on cell volume. The higher NaCl concentration decreases the maximal cell volume but also causes the smaller final volume of cells. In order to restore RBC volume, the isotonic dialysate is necessary to be used in the subsequent washing process (The length of tubes is set to be 100 times of the hollow fiber length in simulation; in Fig.5, β was $7.5 \times 10^9/s$ and the initial hematocrit was 16.25%).

4.5 Effects of blood and dialysate flow rates on mass transfer

For the given dialysate and ultrafiltration, the choice of blood and dialysate flow rates is very crucial for reducing the osmotic damage of RBCs. Both increasing blood flow rates and decreasing dialysate flow rates can reduce the maximal volume of RBCs. Fig.6 shows maximal cell volumes under different pairs of blood and dialysate flow rates. For the given simulative condition in this paper, only those pairs of blood and dialysate flow rates located in the triangular “safe zone” are ideal (RBC expansion limit is about twice of its isotonic volume). By drawing such figures under different operation conditions, one could find the suitable blood and dialysate flow rates to control cell volume in the range of expansion. In addition, for any given hypertonic dialysate, one must be careful of the minimal cell volume. One could first calculate the final cell volume at equilibrium according to the outlet condition of RBCs and then check whether the minimal cell volume is lower than the shrinkage limit of RBCs. If so, the operation condition of blood and dialysate flow rates still cannot be used.

5. Conclusions

As a complement to the unsteady-state model presented in our previous study, a steady-state model coupling mass transfers across cell and fiber membranes is developed to study the removal of CPAs from cryopreserved blood with hollow fiber modules. This model is validated by the experiments with various operation conditions and compared with the unsteady-state model on cell volume change along axial direction and CPA concentration variation at the blood outlet. Then the model is used to study the effects of ultrafiltration flow rates, NaCl concentrations in dialysate, blood flow rates and dialysate flow rates on mass transfer.

The simulative results show that the increase of ultrafiltration flow rates can not only reduce the osmotic damage of RBCs but also increase the clearance of CPAs. The usage of hypertonic dialysate is another method to reduce the osmotic damage of RBCs, but it could lead to hypertonic damage/excessive shrinkage of RBCs. By drawing contour figures of normalized RBC volume under different operation conditions, one could easily find the suitable blood and dialysate flow rates to control cell volume variation in the limit of expansion.

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List of Symbols

A_c	Cell membrane area (μm^2)
J_n	Non-permeable solute flux across unit fiber membrane area ($\text{mol}/\mu\text{m}^2/\text{s}$)
J_s	Permeable solute flux across unit fiber membrane area ($\text{mol}/\mu\text{m}^2/\text{s}$)
J_v	Volume flux across unit fiber membrane area ($\mu\text{m}^3/\mu\text{m}^2/\text{s}$)
L	Effective length of fibers (cm)
L_p	Hydraulic permeability ($\mu\text{m}/\text{min}/\text{atm}$)
m	Concentration of solutes ($\text{mol}/\text{kg H}_2\text{O}$)
N	Number of hollow fibers
P_b	Pressure of blood side (Pa)
P_d	Pressure of dialysate side (Pa)
P_n	NaCl permeability (cm/min)
P_s	CPA permeability (cm/min)
Q	Flow rate (ml/min)
R	Hollow fiber radius (μm)
r_M	Module inner radius (cm)
R	Universal gas constant (kcal/mol/K)
T	Absolute temperature (K)
V	Volume (μm^3)
V_{bc}	Osmotically inactive cell volume (μm^3)
V_{bp}	Percentage of osmotically inactive cell volume at the isotonic condition
V_{c0}	Initial cell volume (μm^3)
V_{iso}	Isotonic volume of cells (μm^3)
$\bar{V}_s$	Partial molar volume of permeable solute (l/mol)

Greek letters

γ	Cell density (number/ μl)
β	Cell number per unit time (number/s)
μ	Viscosity ($\text{N}\cdot\text{s}/\text{m}^2$)
σ	Reflection coefficient (0~1)

Subscripts

$1, 2, 3$	1 , inside of cells; 2 , outside of cells but inside of fibers 3 , outside of fibers
c, h	c , cell membrane; h , hollow fiber membrane
b, d	b , lumen side (inside of fibers); d , shell side (outside of fibers)
i, o	i , inner; o , outer
w	water

Superscript

0	Previous time
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Fig.1.
Schematic of removing CPAs with a hollow fiber module

Fig.2.
Schematic of control volume

Fig.3. Comparisons of cell volumes and CPA concentrations calculated by steady-state and unsteady-state models

Fig.4.
Effects of ultrafiltration flow rates on cell volume and CPA concentration

Fig.5.
Effects of hypertonic NaCl dialysates on cell volume

Fig.6.
Maximal cell volumes under different blood and dialysate flow rates

Table 1

Boundary Conditions

Parameters			
Pressure		$P_b(L)=P_{bout}$	$P_d(0)=P_{dout}$
Flow Rate	$Q_b(L)=Q_{bout}$	$Q_b(0)=Q_{bin}$	$Q_d(L)=Q_{din}$
Glycerol Concentration	$m_{s,1}(0)=m_{b,s,in}$	$m_{s,2}(0)=m_{b,s,in}$	$m_{s,3}(L)=0$
NaCl Concentration	$m_{n,1}(0)=m_{b,n,in}$	$m_{n,2}(0)=m_{b,n,in}$	$m_{n,3}(0)=m_{b,n,in}$
Cell Volume	$V_c(0)=V_{co}$		

Table 2

Parameters used in this paper

Parameters	Units	Values
Hydraulic permeability of cell membrane, $L_{p,c}$	m/s/Pa	1.74×10^{-12} ▲
Solute permeability of RBC membrane, $P_{s,c}$	m/s	6.61×10^{-8} ▲
Reflection coefficient of cell membrane, σ_c	-	0.841▲
Human RBC membrane area, A_c	μm^2	135▲
Isotonic volume of human RBCs, V_{iso}	μm^3	98.3▲
Module inner radius, R_M	cm	0.021☼
Effective length of fibers, L	cm	21.5☼
Hollow fiber inner radius, r_i	μm	107.5☼
Hollow fiber outer radius, r_o	μm	157.5☼
Total membrane area, A_{total}	m^2	1.4☼
Partial molar volume of glycerol, V_s	l/mol	7.1×10^{-2} *

* Data from the literature [25];

▲ Data from the literature [26];

☼ Data from Polyflux™ 6LR (Gambro Co.)

Validation of steady-state model under different NaCl concentrations at dialysate inlet, different ultrafiltration flow rates, and different blood and dialysate flow rates (Unit: mOsm)

Table 3

No.	Q_{bin}	Q_{din}	Q_{uf}	$m_{b,in}$	$m_{b,n,in}$	$m_{d,n,in}$	$m_{b,s,out}+m_{b,n,out}$		$m_{d,s,out}+m_{d,n,out}$	
							Exp.	Num.	Error	
1	252.575	536.325	27.175	1084.5	281.0	0	376.2	375.0	0.32%	428.0
2	252.575	536.325	27.175	1084.0	283.0	276.5	601.2	599.9	0.22%	614.2
3	252.575	536.325	27.175	1095.0	281.0	564.0	854.0	836.8	2.02%	798.8
4	252.575	536.325	27.175	1092.0	280.5	846.0	1065.0	1066.0	0.09%	980.0
5	252.575	579.725	21.275	1092.0	274.0	274.0	602.2	593.6	1.43%	575.2
6	252.575	641.050	15.350	1083.7	278.0	278.0	601.8	589.3	2.08%	549.2
7	306.685	536.325	38.238	1089.0	275.0	275.0	712.0	681.9	4.23%	630.2
8	370.175	536.325	51.950	1090.5	272.5	272.5	791.2	762.6	3.61%	662.8