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# Permeation of blood cells from umbilical cord blood through surface-modified polyurethane foaming membranes

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ABSTRACT

Blood cell permeation of umbilical cord blood (UCB) through surface-modified foamed polyurethane membranes having carboxylic group (PU-COOH) with different pore sizes (i.e., 5, 8.9 and 12 μm) was investigated. Red blood cells and platelets can permeate through PU-COOH membranes with any pore sizes analyzed, while leukocytes and hematopoietic stem and progenitor cells (HSPCs) cannot permeate through PU-COOH membranes with permeation ratio of less than 5%. Several recovery solutions (e.g., 20% dextran, 0.5 wt% serum albumin solution, plasma solution) were evaluated for the isolation of several blood cells including HSPCs from PU-COOH membranes after UCB permeated through the membranes. Recovery ratios of leukocytes were relatively low through PU-COOH membranes having pore size ($r$) = 5 μm, while higher recovery ratio of RBCs was observed than that of leukocytes. The recovery ratio of HSPCs was found to be 8–27% depending on the membrane pore sizes and recovery solution. The recovery ratio of HSPCs from UCB (8–27% in this study) was found to be less than that from peripheral blood reported previously (60–80% [A. Higuchi, M. Sekiya, Y. Gomei, M. Sakurai, W.-Y. Chen, S. Egashira, Y. Matsuoka, Separation of hematopoietic stem cells from human peripheral blood through modified polyurethane foaming membranes, J. Biomed. Mater. Res.: A 85A (4) (2008) 853–861]). This suggests that the purification of HSPCs form UCB is significantly difficult compared to that from peripheral blood, because of high expression of adhesion proteins of HSPCs in UCB.



## 1. Introduction

Efficient cell separation is an important issue for the successful isolation and purification of blood cells, stem cells and specific tissue cells. Centrifugation [1,2], affinity column chromatography [3,4], fluorescence activated cell sorting (FACS) [5,6], magnetic cell selection (MACS) [7,8], and membrane filtration [9–14] are techniques typically employed for cell separation. Highly purified cellular preparations are obtained using Ficoll–Paque method followed by FACS or MACS in conjunction with a fluorescently labeled antibody as the cell-surface marker, while the cell separation by membrane filtration method is less specific, but is simple and easy operation, and sterility can maintain during the process. Only half an hour is necessary to purify hematopoietic stem and progenitor cells (HSPCs) from peripheral blood by the membrane filtration method, while the conventional Ficoll–Paque followed by magnetic cell sorting (MACS) method needs around 8 h.

HSPCs are the "mother cells" of blood, capable of producing several kinds of differentiated blood cells such as red blood cells, platelets, and white blood cells. HSPCs bear the CD34 cell surface marker [15], and are thought to be responsible for hematopoiesis [15]. Therefore, the transplantation of $CD34^+$ cells is required in the therapy of patients with acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndromes, and systemic mastocytosis [16]. In previous investigations [9–11], cell separation from only peripheral blood was investigated using surface-modified polyurethane (PU) membranes, carrying different functional groups. $CD34^+$ cells from peripheral blood adhered more strongly to the membranes than red blood cells, platelets, T cells or B cells [9]. Therefore, purified $CD34^+$ cells from peripheral blood were obtained in the recovery solution when this solution was passed through the membranes after permeation of peripheral blood.

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However, cell separation from umbilical cord blood (UCB) by membrane filtration method has not yet reported in our knowledge. There exist 15–75 times higher number of HSPCs in umbilical cord blood than that in peripheral blood. Furthermore the population and concentration of blood cells and plasma proteins are quite different in the blood solution from peripheral blood and UCB. It is expected that cell permeation of UCB should be quite different from that of peripheral blood through the membranes. Therefore, in this study we investigate blood cell permeation of UCB through surface-modified PU membranes by the membrane filtration method to obtain fundamental knowledge of blood cell permeation of UCB before the development of the optimized membranes for HSPC purification. We will compare and discuss the permeation ratio and recovery ratio of several blood cells through the membranes from peripheral blood reported previously [9–11] and those obtained from UCB investigated in this study.

## 2. Materials and methods

### 2.1. Materials

Base membranes used for the chemical modification were polyurethane (PU) foaming membranes (Ruby Cell S, Toyo Polymer Co., Ltd.) and PU foaming membranes containing 0.61% of epoxy group (PU-epoxy), which were plasma-polymerized using glycidyl methacrylate on the PU foaming membranes. The average pore size of the PU and PU-epoxy membranes evaluated from Capillary Flow Porometer measurements (Porous Materials Inc.) was 5.2, 8.9 and 12 μm. The PU and PU-epoxy membranes had 86% porosity and 1.2 mm thickness. Human serum albumin (HSA, 019-10503, Wako Pure Chemical Industries Ltd.) and dextran 40,000 (049-22331, Wako Pure Chemicals Industries Ltd.) were used as received. Anti-glycophorin A antibody conjugated with PE (phycoerythrin) (IM2211, Beckman Coulter Co.), anti-CD34 antibody conjugated with PE (A07776, Beckman Coulter Co.), and anti-CD45 antibody conjugated with FITC (IM2653K, Beckman Coulter Co.) were used as received. 7-AAD (A07704, Beckman Coulter Co.), Optilyse C (IM1401, Beckman Coulter Co.) and Flow-count beads solution (7547053, Beckman Coulter Co.) were also used as received. Other chemicals, purchased from Tokyo Chemical Co., were reagent grade and were used without further purification. Ultrapure water produced from Milli-Q System (Millipore Corporation) was used throughout the experiments.

### 2.2. Preparation of surface-modified PU foaming membranes

Carboxylic acid group was introduced from opening reaction of epoxy group on the PU-epoxy membranes [9–11]. PU-epoxy membranes were immersed in 0.5 mol/L of glycine solution containing 0.1 mol/L of NaOH at 353 K for 24 h. The anticipated product by the ring-opening reaction of epoxy group is shown in Fig. 1. The resultant membranes were referred to as PU-COOH membranes. After the reaction, the membranes were rinsed in ultrapure water for 3 h, and stocked in ultrapure water at 4 °C.

### 2.3. Blood cell permeation

UCB was collected into a blood bag with an informed concept. The blood (feed solution) was subsequently injected into the Syringe 2 described in a previous study [9–11]. Two syringes (syringe 1 and syringe 2) were attached by head to head. Water was injected into syringe 1 using perister pump (MP-3, Tokyo Rikakiki Co.) at the speed of 0.3 mL/min, and therefore, head of syringe 1 pushed back syringe 2 containing blood subsequently. The blood in syringe 2 was, therefore, filtered through PU-COOH membranes attached inside the membrane holder at the filtration rate of 0.3 mL/min and 25 °C. The number of cells in the permeate and feed solutions ($N_p$ and $N_f$, respectively) was counted from the flow cytometry (Coulter EPICS$^{TM}$ XL, Beckman-Coulter Co.) as described as the following section. The permeation ratio is defined as:

$$\text{permeation ratio}(\%) = \left(\frac{N_p}{N_f}\right) \times 100 \quad (1)$$

After the blood filtration, the membranes were upside down inside the membrane holder, and a recovery solution (0.5 wt% HSA solution, 20 wt% dextran solution, Platelet-rich plasma [PRP], or Platelet-poor plasma [PPP]) was permeated through the membranes using the same membranes and the apparatus at filtration speed of 1.0 mL/min and 25 °C to remove the adhered cells on the membranes and to collect them in the recovery solution. PRP or PPP was prepared from centrifugation of UCB at 1000 or 2800 rpm for 15 min, respectively.

The recovery ratio is defined as:

$$\text{recovery ratio}(\%) = \left(\frac{N_r}{N_f}\right) \times 100 \quad (2)$$

where $N_r$ is the number of cells in the permeate solution after the permeation of recovery solution.

The permeation experiments of blood were performed on each membrane using three independent membranes, and the number of each specific cell was counted from a flow cytometric analysis as described in the following section and finally averaged.

### 2.4. Flow cytometric analysis of blood cells

The number of red blood cells, platelets and leukocytes in the feed (peripheral blood) and permeate solutions was analyzed from surface markers of glycophorin A for red blood cells, CD41 for platelets and CD45 for leukocytes, respectively. The feed and permeate samples were diluted 10 times. 20 μL of anti-glycophorin A antibody, 20 μL of anti-CD41 antibody and 20 μL of anti-CD45 antibody were added into 100 μL of the above-diluted sample. The sample incubated under dark place for 20 min, after it was agitated using a Vortex mixer (VX-100, Montreal Biotech Inc.) for 1 min. The sample was again diluted 100 times, and 100 μL of flow-count beads solution was added into 500 μL of the diluted sample, subsequently. Finally the sample was analyzed using the flow cytometry, and the number of red blood cells, platelets and leukocytes was counted [9–11].

The number of HSPCs was analyzed by $CD34^+$ cells followed by International Society of Hemathotherapy and Graft Engineering (ISHAGE) guidelines [17] using the flow cytometry after dying the cells with anti-CD34 antibody, anti-CD45 antibody and 7AAD [9–11].

## 3. Results and discussion

### 3.1. Permeation of blood cells through membranes having different pore size

Blood cell permeation of UCB through PU-COOH membranes with different pore sizes (i.e., 5, 8.9 and 12 μm) was investigated. The numbers of specific blood cells (RBCs, leukocytes, platelets and HSPCs) that permeated were evaluated using flow cytometry analysis of surface antigens on the cells bound to each fluorescence-labeled antibody. Fig. 2 shows flow-cytometric scattergrams of blood cells after lysis of red blood cells. HSPCs were analyzed by counting $CD45^+CD34^+$ cells following ISHAGE guidelines [17]. The gating of $CD45^+$ cells on the flow-cytometric scattergrams is shown in Fig. 2(a). Only living cells analyzed with the 7AAD