Separation of Hematopoietic Stem and Progenitor Cells from Human Peripheral Blood Through Polyurethane Foaming Membranes Modified with Several Amino Acids

Akon Higuchi,[1] Siou-Ting Yang,[1] Pei-Tsz Li,[1] Hui Chen,[1] Ruoh-Chyu Ruaan,[1] Wen-Yih Chen,[1] Yung Chang,[2] Yu Chang,[3,4] Eing Mei Tsai,[4] Qing-Dong Ling,[5] Han-Chow Wang,[6] Shih-Tien Hsu[7]

[1]*Department of Chemical and Materials Engineering, National Central University, Jhongli, Taoyuan 32001, Taiwan*
[2]*Department of Chemical Engineering, R&D Center for Membrane Technology, Chung Yuan Christian University, Chungli, Taoyuan 320, Taiwan*
[3]*Department of Obstetrics and Gynecology, Kaohsiung Municipal Hsaio-Kang Hospital, Hsiao-Kang District, Kaohsiung 812, Taiwan*
[4]*Department of Obstetrics and Gynecology, College of Medicine, Kaohsiung Medical University, Kaohsiung,Taiwan*
[5]*Cell Biology and Anatomy Laboratory, Cathy Madical Research Institute, Cathy General Hospital, Hsi-Chi City, Taipei 221, Taiwan*
[6]*Obstetrics & Gynecology Division, Li Shin Hospital, Pingjen City, Tao-Yuan County 32405, Taiwan*
[7]*Department of Community Medicine, Li Shin Hospital, Pingjen City, Tao-Yuan County 32405, Taiwan*

Received 3 December 2008; accepted 5 April 2009
DOI 10.1002/app.30564
Published online 8 June 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The separation of hematopoietic stem and progenitor cells ($CD34^+$ cells) from peripheral blood was investigated using foamed polyurethane (PU) membranes modified with several amino acids. $CD34^+$ cells were collected by first allowing the blood to permeate through the membranes, and then passing the recovered solution through the membranes. Optimal conditions for the separation of $CD34^+$ cells were investigated. The highest recovery ratio of $CD34^+$ cells was obtained using three sheets of PU membranes having carboxylic acid groups (PU—COOH) modified with glycine, the membranes having been pretreated by immersion in phosphate buffer solution prior to permeation of blood. A high recovery ratio of $CD34^+$ cells was achieved in a recovery process using 0.5 wt % human serum albumin (HSA) or 20% dextran solution passed through PU—COOH membranes. The recovery ratios of $CD34^+$ cells using platelet-poor plasma and platelet-rich plasma were approximately 20% and 30%, respectively, significantly less than the ratio found using 0.5 wt % HSA solution. Surface-modified membranes having carboxylic acid groups showed a higher recovery ratio of $CD34^+$ cells than membranes having zwitterionic groups. The effect of carboxylic acid groups on the surface-modified PU membranes was to generate weak interactions by electrostatic repulsion between $CD34^+$ cells and the membranes because of the negatively charged surfaces of the cells, allowing them to be detached from the membranes and collected in the recovered solution. J Appl Polym Sci 114: 671–679, 2009

Key words: biomaterials; bioengineering; polyurethanes; separation techniques; hematopoietic stem cells

INTRODUCTION

Membrane technology plays an important role in medical applications in the fields of drug delivery,[1,2] artificial organs,[3] tissue engineering,[4] diagnostic devices,[5] and blood cell separation.[6–9] Efficient cell separation is an important issue for the successful isolation and purification of blood cells, stem cells, and specific tissue cells. Centrifugation,[10,11] affinity column chromatography,[12,13] fluorescence-activated cell sorting (FACS),[14,15] magnetic cell selection (MACS),[16,17] and membrane filtration[7–9,18–20] are techniques typically employed for cell separation. Highly purified cellular preparations are obtained using FACS or MACS in conjunction with a fluorescently labeled antibody as the cell-surface marker, whereas the centrifugal separation of cells is commonly used to isolate platelets, leukocytes, mononuclear cells, red blood cells, and non-blood cells.

Cell separation through membrane filtration has been reported by several researchers.[7–9,18–22] Blood for transplantation is typically passed through

Correspondence to: A. Higuchi (higuchi@ncu.edu.tw).
Contract grant sponsor: National Science Council of Taiwan; contract grant numbers: NSC96-2218-E-008-007, 97-2221-E-008-011-MY3 and NSC97-2120-M-008-002.
Contract grant sponsor: VGHUST Joint Research Program, Tsou's Foundation; contract grant numbers: VGHUST97-P3-08 and VGHUST98-P3-11.

membrane filters (leukocyte removal filters) in order to eliminate leukocytes, which function to prevent infection by viruses such as the human immunodeficiency virus and the hepatitis C virus.[21,22] Compared with other cell separation methods, membrane filtration is simple and inexpensive, and sterility is easy to maintain during the process.

Hematopoietic stem cells 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. Hematopoietic stem and progenitor cells bear the CD34 cell surface marker,[23] and are thought to be responsible for hematopoiesis.[23] 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.[24] In previous investigations,[7–9] cell separation from peripheral blood was investigated using surface-modified polyurethane (PU) membranes with a pore size of 5 μm, carrying different functional groups. $CD34^+$ cells adhered more strongly to the membranes than red blood cells, platelets, T cells, or B cells.[7] Therefore, purified $CD34^+$ cells were obtained in the solution recovered from 0.5 wt % human albumin (HSA) solution when this was passed through the membranes after permeation of peripheral blood. We also investigated blood cell permeation through unmodified PU and PU membranes carrying carboxylic acid groups (PU—COOH) with pore sizes of either 5.2 or 12 μm at different blood permeation speeds.[8] The most highly purified $CD34^+$ cells (highest recovery ratio of $CD34^+$ cells to red blood cells) were obtained in a recovery process using PU—COOH membranes with pore size $r = 5.2$ μm at blood permeation speeds of 0.3 mL/min to 1 mL/min.[8] We also developed different kinds of filtration membranes such as Pluronic-coated and Pluronic-immobilized membranes.[9] $CD34^+$ cells were efficiently recovered (approximately 80%) through PU—COOH membranes in a process using 20 wt % aqueous dextran as the recovery solution at 4°C.

Optimal conditions for the purification of $CD34^+$ cells from blood using membrane filtration were still undetermined. In this study, we prepared not only the PU—COOH membranes used in our previous studies,[7–9] but also different types of PU—COOH membranes modified with several amino acids, and conducted further experiments on the separation of $CD34^+$ cells using these modified membranes. Furthermore, we used a variety of experimental conditions in the permeation and recovery processes, and investigated the effect of permeation volume, type, and concentration of recovery solution on the detachment of $CD34^+$ cells (i.e., recovery ratio of $CD34^+$ cells) during the recovery process at 25°C.

MATERIALS AND METHODS

Characterization of membranes

The average pore size of the PU and PU-epoxy membranes was evaluated from capillary flow porometer (Porous Materials Inc.). Scanning electron microscope (SEM) were performed by JEOL 255-II (Jeol Co.).

Materials

Base membranes used for the chemical modification were polyurethane (PU) foaming membranes (Imugard III-RC, Terumo Corporation) and PU foaming membranes containing 0.61% of epoxy group (PU-epoxy), which were plasma-polymerized using glycidyl methacrylate on the PU foaming membranes. Human serum albumin (HSA, 019-10503, Wako Pure Chemical Industries Ltd.), bovine serum albumin (BSA, A3059-50G55, Sigma-Aldrich, Co.) 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-CD3 antibody conjugated with PE (IM1282, Beckman Coulter Co.), anti-CD4 antibody conjugated with PE (627808-300, Beckman Coulter Co.), anti-CD8 antibody conjugated with PE (IM1383, Beckman Coulter Co.), and anti-CD19 antibody conjugated with FITC (IM1284, Beckman Coulter Co.), anti-CD41 antibody conjugated with FITC (fluorescein isothiocyanate)(IM0649, Beckman Coulter Co.), and anti-CD45 antibody conjugated with FITC (IM2653K, Beckman Coulter Co.) were used as received. Stem-kit™ reagents (IM3630, 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 Academic A10 System (Millipore Corporation) was used throughout the experiments.

Preparation of surface-modified PU foaming membranes

Several type of functional groups were introduced from opening reaction of epoxy group on the PU-epoxy membranes, which were following by the reaction between epoxy group and several amino acids (i.e., glycine (Gly), aspartate (Asp), asparagine (Asn), lysine (Lys), and arginine (Arg)). PU-epoxy membranes were immersed in 0.5 mol/L of amino acid 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 Figure 1. The resultant membranes were referred to as

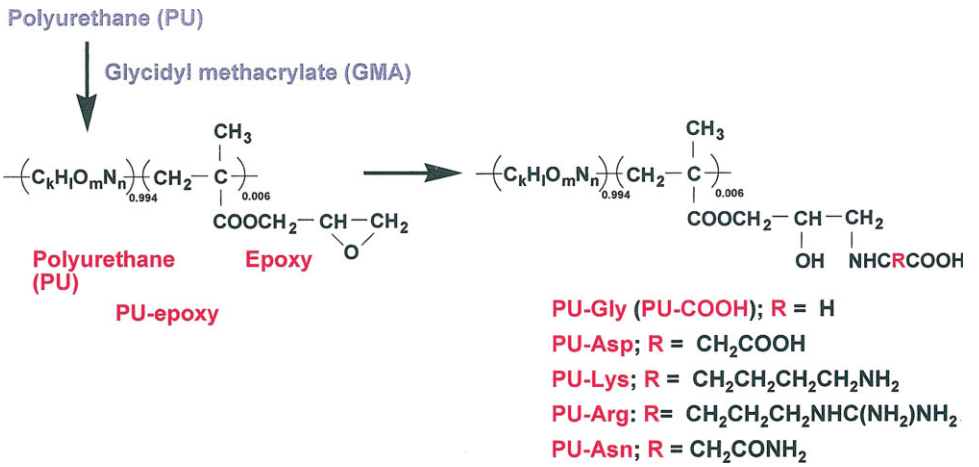

Figure 1 Reaction scheme for synthesizing PU-Gly (PU—COOH), PU-Asp, PU-Lys, PU-Arg, and PU-Asn membranes. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

PU-Gly (PU—COOH), PU-Asp, PU-Asn, PU-Lys, and PU-Arg membranes. After the reaction, the membranes were rinsed in ultrapure water for 3 h, and stocked in ultrapure water at 4°C.

Blood cell permeation

A sample of 35 mL of human fresh blood (man or woman, 22–24 years old) was collected with an informed concept using vacuum tubes (7 mL, Venoject II, Terumo, Co.) containing 10.5 mg of EDTA·2Na. The blood (feed solution) was subsequently injected into the Syringe 2 described in a previous study.[7–9] 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 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

$$\text{Permeation ratio}(\%) = (N_p/N_f) \times 100 \qquad (1)$$

After the blood filtration, the membranes were upside down inside the membrane holder, and a recovery solution (PBS, 0.05–3 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 blood, which was obtained with an informed concept, at 1000 rpm or 2800 rpm for 15 min, respectively.

The recovery ratio is defined as

$$\text{Recovery ratio}(\%) = (N_r/N_f) \times 100 \qquad (2)$$

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

The membranes used in the blood filtration were unmodified PU, PU-Gly (PU—COOH), PU-Asp, PU-Asn, PU-Lys, and PU-Arg membranes. 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.

Flow cytometric analysis of blood cells

The number of red blood cells, platelets, and lymphocytes 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 lymphocytes, respectively. The feed and permeate samples were diluted 10 times. Twenty microliters 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,

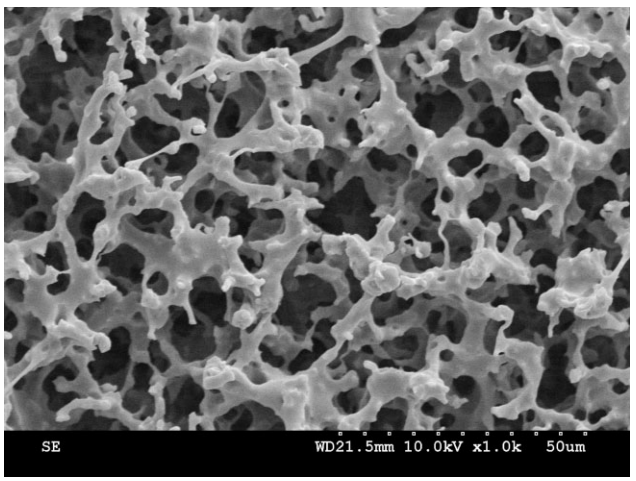

Figure 2 Scanning electron micrograph of surface of PU membranes.

and the number of red blood cells, platelets, and lymphocytes was counted.[7–9]

The number of T and B cells in the feed (peripheral blood) and permeate solutions was analyzed from surface markers of CD3 and CD19, respectively. The number of helper T cells and suppressor T cells in the feed (peripheral blood) and permeate solutions was analyzed from surface markers of CD4 and CD8, respectively. The analytical procedures to obtain the number of T cells, B cells, helper T cells, and suppressor T cells were followed as the same procedures of the analysis of red blood cells and platelets except using different antibodies.

The number of hematopoietic stem and progenitor cells was analyzed by $CD34^+$ cells followed by ISHAGE (International Society of Hemathotherapy and Graft Engineering) guidelines[25,26] using Stem-Kit™ (Beckman Coulter Co.) and the flow cytometry.[7–9]

RESULTS

Membrane characterization

The average pore size of the PU and PU-epoxy membranes was evaluated to be 5.2 μm from capillary flow porometer measurements. The PU and PU-epoxy membranes had 86% porosity and 1.2 mm thickness. Figure 2 shows a surface of unmodified PU membranes measured by a SEM. Irregular pore size and pore structure were observed on the PU membranes. No apparent difference of the membrane pore size and structure was found on PU, PU-epoxy, and PU membrane modified with several amino acids (data not shown).

Effect of number of membrane sheets on permeation of $CD34^+$ cells

We first investigated the effect of the number of membrane sheets on the permeation of hematopoietic stem and progenitor cells ($CD34^+$) through unmodified (PU) membranes and membranes modified with glycine, carrying carboxylic acid groups (PU-Gly or PU—COOH, Fig. 1). The number of $CD34^+$ cells was evaluated using flow cytometric analysis of surface antigens on the cells bound to each fluorescence-labeled antibody.

Figure 3 shows flow-cytometric scattergrams of blood cells after lysis of red blood cells. Hematopoietic stem and progenitor cells were analyzed by counting $CD45^+$ $CD34^+$ cells following ISHAGE guidelines.[25,26] The gating of $CD45^+$ cells on the flow-cytometric scattergrams is shown in Figure 3(a). Only living cells analyzed with the 7AAD dye exclusion method were counted in this study. Cells with high expression of CD34 were counted as hematopoietic stem cells, as shown in Figure 3(b).

Figure 4 shows the permeation and recovery ratios of $CD34^+$ cells through one or three sheets of PU or

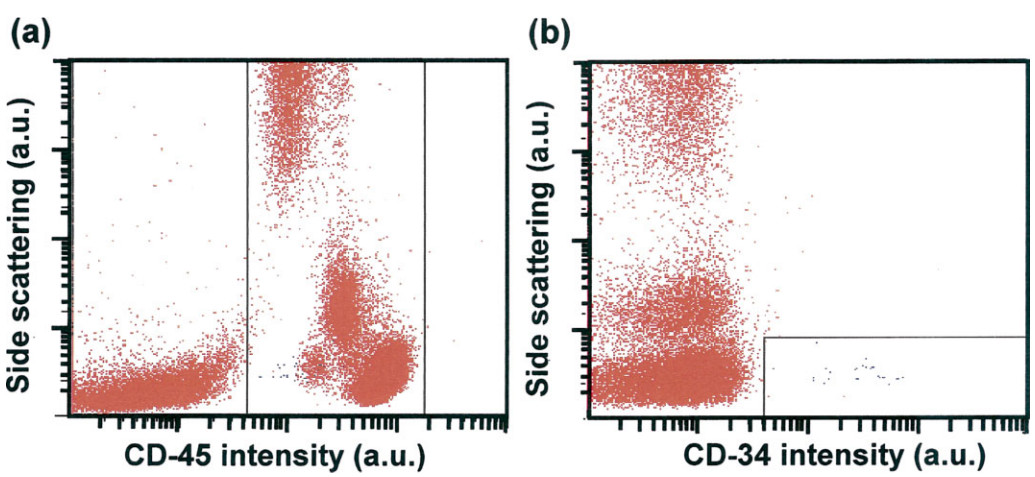

Figure 3 Flow-cytometric scattergrams of blood dyed with anti-CD45 antibody conjugated with FITC (a) and anti-CD34 antibody conjugated with PE (b) in the fluorescence intensity and light intensity of side scattering. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

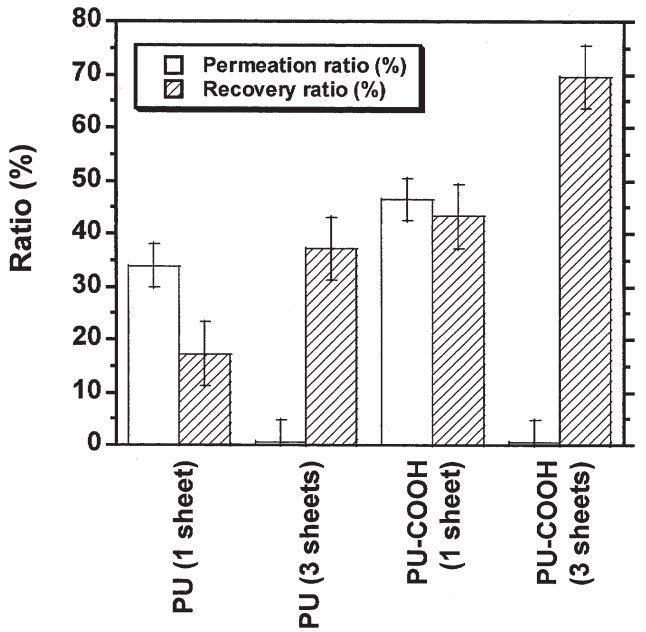

Figure 4 Effect of number of membrane sheets (one or three sheets) on permeation and recovery ratios of $CD34^+$ cells through PU and PU—COOH membranes after permeation of peripheral blood at 25°C. Data are expressed as the means ± S.D. of three independent measurements.

PU—COOH membranes. It was found that $CD34^+$ cells could leak through (permeation ratio > 30% when 6 mL of peripheral blood permeated) one sheet of PU or PU—COOH membranes, whereas these cells could not be detected in the permeate solution when three sheets of PU or PU—COOH membranes were used. Three sheets of PU membranes were found to be sufficient to trap most of the $CD34^+$ cells in 6 mL of peripheral blood. A higher recovery ratio was found for three sheets of PU or PU—COOH membranes than for one sheet, because a higher number of $CD34^+$ cells adhered to three membrane sheets than to one sheet. Therefore, three sheets of PU and surface-modified PU membranes were used for the blood permeation experiments in the following study.

The dependence of permeation and recovery volume on the permeation and recovery ratio of $CD34^+$ cells was investigated, and the results are shown in Figure 5. It was found that the recovery ratio of $CD34^+$ cells decreased with increasing volume of recovery solution. It was found that 4 mL of recovery solution were sufficient to recover $CD34^+$ cells by detaching the adhered cells from the membranes, although 6 mL of recovery solution were actually used in this study.

Effect of membrane pretreatment on recovery ratio of $CD34^+$ cells

Unmodified PU and PU—COOH membranes were immersed in pure water, 0.5 wt % HSA solution or PBS solution for 10 min at 25°C before the permeation of blood to investigate the effect of this pretreatment of the membranes on the permeation of blood cells. Figure 6 shows the permeation ratio (left side on the figure) and recovery ratio (right side on the figure) of $CD34^+$ cells from unmodified PU and PU—COOH membranes. The permeation ratio for both membranes was less than 10% after any treatment of the membranes in this study, though the membranes treated with PBS tended to show a higher recovery ratio of $CD34^+$ cells than those treated with pure water. The pH of pure water and HSA solutions was less than 6, whereas the PBS was adjusted to pH 7.4. Therefore, the initial pH of the membranes probably affected the permeation of $CD34^+$ cells.

Effect of recovery solution on recovery ratio of $CD34^+$ cells

A 0.5 wt %.solution of HSA was mainly used as a recovery solution to obtain $CD34^+$ cells from blood in our previous study.[7–9] This recovery solution was used as a detergent to detach cells bound to membranes in these experiments. Several other recovery solutions (e.g., 20% dextran, 0.05–3 wt % HSA solution, PRP, and PPP) were also evaluated for the isolation of $CD34^+$ cells from blood.

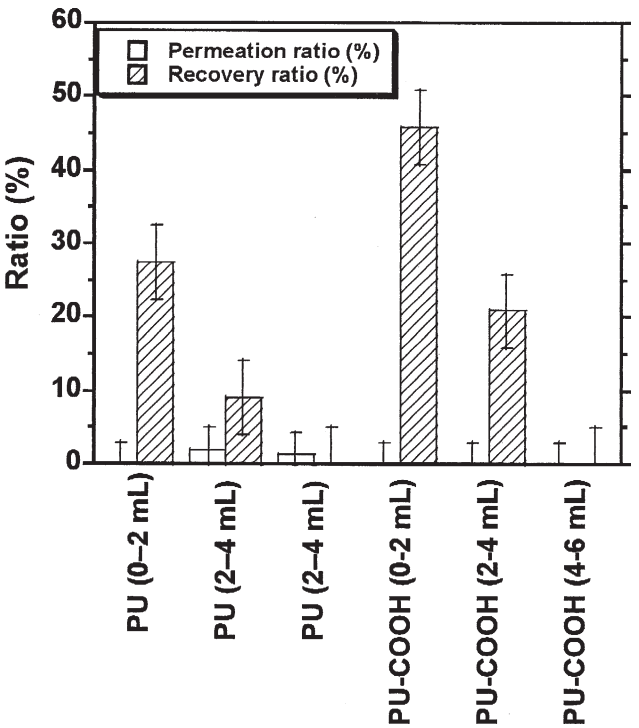

Figure 5 Dependence of permeation and recovery volume on the permeation and recovery ratios of $CD34^+$ cells through unmodified PU and PU—COOH membranes after permeation of peripheral blood at 25°C. Data are expressed as the means ± S.D. of three independent measurements.

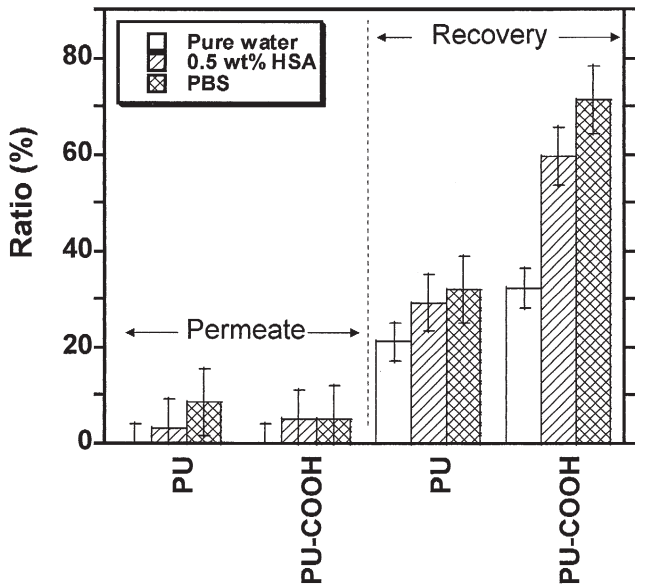

Figure 6 Permeation and recovery ratios of $CD34^+$ cells through unmodified PU and PU—COOH membranes after permeation of peripheral blood at 25°C. The membranes were pretreated for 10 min at 25°C by immersion in pure water, 0.5 wt %.HSA solution or PBS (pH 7.4) before permeation of peripheral blood. Data are expressed as the means ± S.D. of three independent measurements.

Figure 7 shows the recovery ratios of $CD34^+$ cells from PU—COOH membranes when PBS, 0.05 wt % HSA, 0.5 wt % HSA, 3 wt % HSA, PPP, and PRP solutions were used for recovery at 25°C. When HSA solution was used as the recovery solution, the optimal concentration of HSA for the highest recovery ratio of $CD34^+$ cells was 0.5 wt %. A high recovery ratio of $CD34^+$ cells was obtained when 20 wt % dextran or 0.5 wt % HSA was used as the recovery solution. The recovery ratios of $CD34^+$ cells using PPP and PRP were approximately 20% and 30%, respectively, significantly less than for 0.5 wt % HSA. However, if $CD34^+$ cells purified using this membrane filtration method were destined for direct transplantation, cells in PRP or PPP might be more useful than cells in dextran or HSA, because these cells can be directly cultured in PRP or PPP.

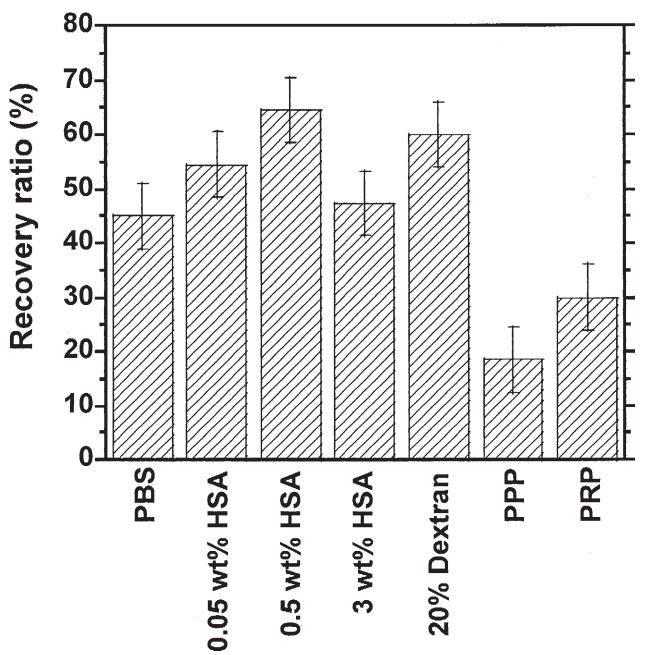

Figure 7 Recovery ratio of $CD34^+$ cells through PU—COOH membranes after permeation of peripheral blood at 25°C. PBS, 0.05 wt % HSA, 0.5 wt % HSA, 3 wt % HSA, PPP, or PRP solutions were used as the recovery solution at 25°C. Data are expressed as the means ± S.D. of three independent measurements.

Separation of various blood cells

The permeation and recovery ratios of several types of blood cells through unmodified PU and PU—COOH membranes were analyzed. The blood cells examined were red blood cells, platelets, T ($CD3^+$) cells, helper T ($CD4^+$) cells, suppressor T ($CD8^+$) cells, B ($CD19^+$) cells, and $CD34^+$ cells. The permeation ratios measured for these membranes are shown in Figure 8. Red blood cells permeated relatively easily through unmodified PU and PU—COOH membranes—their permeation ratio was higher than 50%. Platelets also permeated through the membranes more easily than several types of lymphocytes. T cells, helper T cells, suppressor T cells, and B cells did not permeate (permeation ratio $< 5\%$) through either unmodified PU or PU—COOH

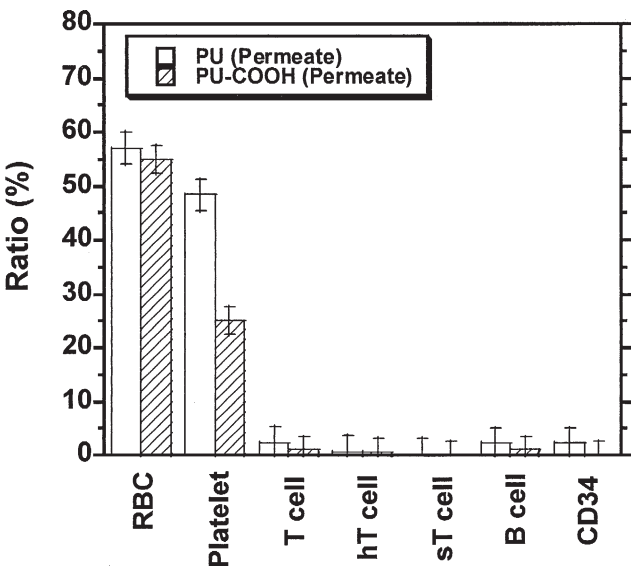

Figure 8 Permeation ratios of red blood cells (RBC), platelets, T cells, helper T (hT) cells, suppressor T (sT) cells, B cells, and $CD34^+$ cells through unmodified PU and PU—COOH membranes after permeation of peripheral blood at 37°C. Data are expressed as the means ± S.D. of three independent measurements.

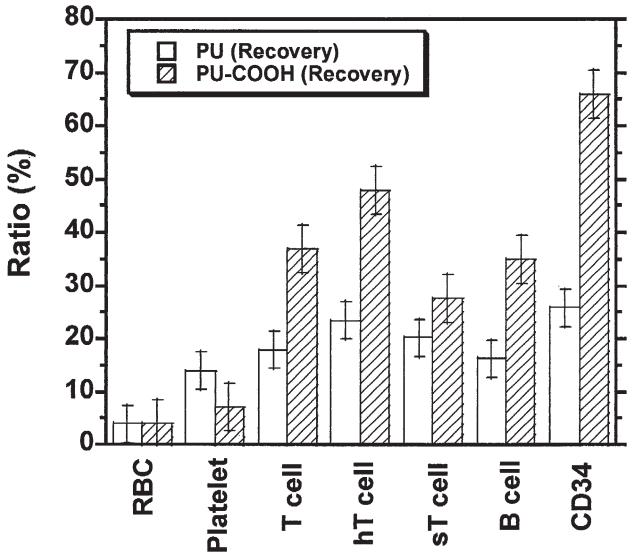

Figure 9 Recovery ratios of red blood cells (RBC), platelets, T cells, helper T (hT) cells, suppressor T (sT) cells, B cells, and $CD34^+$ cells through unmodified PU and PU—COOH membranes after permeation of peripheral blood at 37°C. Data are expressed as the means ± S.D. of three independent measurements.

membranes, indicating that T and B cells adhered strongly to these membranes. Figure 9 shows the recovery ratios for the various cells. The recovery ratios of red blood cells and platelets through unmodified PU and PU—COOH membranes were found to be low (i.e., <20%). This was because most red blood cells and platelets permeated through the membranes. Recovery ratios measured for T cells, helper T cells, suppressor T cells and B cells using PU—COOH membranes were higher than for red blood cells and platelets. Although the recovery ratio of helper T cells was found to be slightly higher than that of suppressor T cells, the separation of T cells from B cells, and of the several kinds of T cells was not expected to be highly efficient because of their similar recovery ratios. This indicated that B cells and the various T cells had similar adhesiveness, likely due to similar expression of adhesive molecules on their surfaces.

Permeation and recovery of $CD34^+$ cells through different PU membranes

Several types of surface-modified PU membranes were also prepared in this study. PU membranes modified with arginine (PU-Arg) and lysine (PU-Lys) had zwitterionic groups, whereas PU membranes modified with glycine (PU-Gly), asparagine (PU-Asn), and aspartate (PU-Asp) had negatively charged carboxylate groups (Fig. 1). PU-Gly is the membrane (PU—COOH) used in our previous study[7–9] and in the previous section of the current investigation. Experiments on the purification of $CD34^+$ cells from peripheral blood were performed using several types of these surface-modified PU membranes, and the permeation and recovery ratios of $CD34^+$ cells through these membranes are shown in Figure 10. The recovery ratio of $CD34^+$ cells from membranes with zwitterionic groups (PU-Arg and PU-Lys) was found to be less than for PU-Gly (PU—COOH) membranes. In our previous study,[7] it was found that the recovery ratio of $CD34^+$ cells was less than 20% through membranes modified with positively charged groups on their surfaces. The presence of the carboxylate groups on the surface-modified PU membranes led to weak electrostatic repulsions between cells and membranes because of the negatively charged surfaces of these cells. The zwitterionic groups on PU-Arg and PU-Lys membranes caused them to have a higher surface potential than PU—COOH membranes, and might therefore have led to the lower recovery ratios of $CD34^+$ cells through the former membranes than through the latter. PU—COOH membranes showed the highest recovery ratio of $CD34^+$ cells, higher than for other negatively charged membranes such as PU-Asp and PU-Asn used in this study.

DISCUSSION

Lineage-negative cells from umbilical cord blood (glycophorin A^-, $CD2^-$, $CD3^-$, CD^-, $CD16^-$, $CD33^-$, $CD38^-$, $CD45^-$, and $CD56^-$) were maintained and found to expand more into primitive hematopoietic stem and progenitor cells than $CD34^+$ or $CD133^+$ cells. These cells also expressed higher levels of the

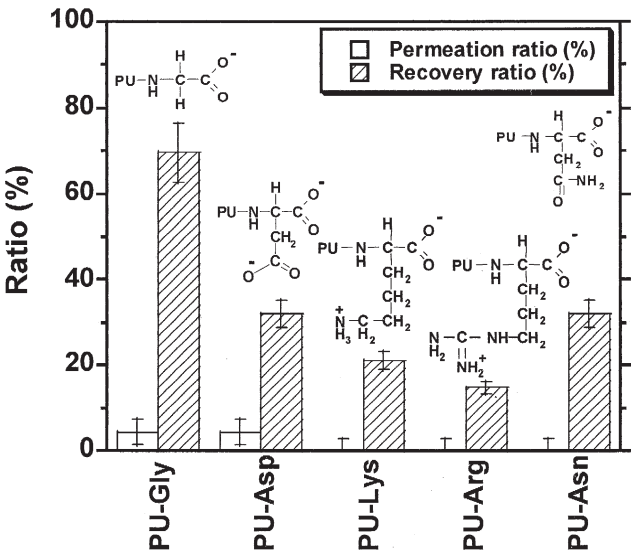

Figure 10 Permeation and recovery ratios of $CD34^+$ cells through surface-modified PU membranes after permeation of peripheral blood at 25°C. Data are expressed as the means ± S.D. of three independent measurements.

cell-adhesion molecules CD162 (expression ratio (ER) = 16.0%) and CD164 (ER = 96.7%) involved in hematopoietic progenitor-forming bone marrow, than CD34 (ER = 14.4%) or CD133 (ER = 7.0%).[27] Therefore, primitive hematopoietic stem and progenitor cells tended to adhere to PU membrane surfaces, due to their expression of these cell-adhesion molecules on their surfaces.

Furthermore, adherent hematopoietic stem and progenitor cells, which expressed adhesion proteins and extracellular matrix components (fibronectin 1, cadherin 11, vascular cell adhesion molecule-1, connexin 43, integrin β-like 1, and TGFBI), reportedly showed higher self-renewing capacity as well as higher long-term hematopoietic culture initiating cell (LTC-IC) frequency.[28] This research strongly suggested that our purified $CD34^+$ cells were more primitive hematopoietic stem and progenitor cells than those obtained from FACS or MACS, because of the adhesion of the cells onto the membranes used for filtration in this study.

Membranes such as PU—COOH, having negatively charged groups, showed higher recovery ratios of $CD34^+$ cells than membranes having net neutral charges. These results indicated that more prevalent weak interactions between $CD34^+$ cells and membrane materials tended to generate a higher recovery ratio of $CD34^+$ cells using these membranes. The CD34 antigen is reported to be a highly sialylated, negatively charged glycophosphoprotein, whose expression decreases as hematopoietic stem and progenitor cells become differentiated.[29–31] Therefore, $CD34^+$ cells having negatively charged surface groups were more weakly attracted to membranes such as PU—COOH, bearing negatively charged groups. This interaction may have led to the higher recovery ratio of $CD34^+$ cells from such membranes.

CONCLUSION

Separation of hematopoietic stem and progenitor cells ($CD34^+$ cells) from peripheral blood was investigated using surface-modified foamed polyurethane (PU) membranes. The highest recovery ratio of $CD34^+$ cells was obtained using three sheets of PU—COOH membranes which had been pretreated by immersion in phosphate buffer solution before permeation of blood. A high recovery ratio of $CD34^+$ cells was achieved in a recovery process using 0.5 wt % human serum albumin or 20% dextran solution, with PU—COOH membranes. The recovery ratios of $CD34^+$ cells using PPP and PRP were approximately 20% and 30%, respectively, and were less than the value found using 0.5 wt % HSA solution. However, if $CD34^+$ cells purified with the membrane filtration method were to be used directly for transplantation, $CD34^+$ cells in PRP or PPP might be more useful than cells in dextran or HSA solution.

The exact surface marker for primitive hematopoietic stem and progenitor cells remains unclear at the current time. Isolating such cells by membrane filtration of umbilical cord or bone marrow is thought to be more effective than MACS or FACS methods, because cell separation in membrane filtration is based not only on cell size, but also on the strength of cell adhesion to the membrane surface. Because it has been suggested that more primitive hematopoietic stem and progenitor cells express negatively charged glycophosphoproteins,[29–31] the purified hematopoietic and stem cells obtained by the present membrane filtration method might have more undifferentiated characteristics than those collected by FACS or MACS. Comparative bioassays, such as the colony forming assay, are now under investigation in our laboratory using hematopoietic and progenitor cells purified by the present membrane filtration method, and also by FACS and MACS.

Of all methods, membrane separation is likely to provide the simplest, cheapest, and most sanitary isolation of primitive hematopoietic stem and progenitor cells.

References

1. Stamatialis, D. F.; Papenburg, B. J.; Gironés, M.; Saiful, S.; Bettahalli, S. N. M.; Schmitmeier, S.; Wessling, M. J Membr Sci 2008, 308, 1.
2. Ma, D.; Mchugh, A. J. J Membr Sci 2007, 298, 156.
3. Dai, W. S.; Barbari, T. A. J Membr Sci 1999, 156, 67.
4. Papenburg, B. J.; Vogelaar, L.; Bolhuis-Versteeg, L. A. M.; Lammertink, R. G. H.; Stamatialis, D.; Wessling, M. Biomaterials 2007, 28, 1998.
5. Lin, L.; Guthrie, J. T. J Membr Sci 2006, 278, 173.
6. Lin, L.; Guthrie, J. T. J Membr Sci 2000, 173, 73.
7. Higuchi, A.; Yamamiya, S.; Yoon, B. O.; Sakurai, N.; Hara, M. J Biomed Mater Res 2004, 68, 34.
8. Higuchi, A.; Iizuka, A.; Gomei, Y.; Miyazaki, T.; Sakurai, M.; Matsuoka, Y.; Hayashi, S. J Biomed Mater Res 2006, 78, 491.
9. Higuchi, A.; Sekiya, M.; Gomei, Y.; Sakurai, M.; Chen, W.-Y.; Egashira, S.; Matsuoka, Y. J Biomed Mater Res 2008, 85, 853.
10. Asahara, T.; Murohara, T.; Sullivan, A.; Silver, M.; Van Der Zee, R.; Li, T.; Witzenbichler, B.; Schatteman, G.; Isner, J. M. Science 1997, 275, 964.
11. Aoki, M.; Yasutake, M.; Murohara, T. Stem Cells 2004, 22, 994.
12. Kikuchi, A.; Karasawa, M.; Tsuruta, T.; Kataoka, K. J Colloid Interface Sci 1993, 158, 10.
13. Ohba, H.; Bakalova, R.; Moriwaki, S.; Nakamura, O. Cancer Lett 2002, 184, 207.
14. Amer, J.; Ghoti, H.; Rachmilewitz, E.; Koren, A.; Levin, C.; Fibach, E. Br J Haematol 2006, 132, 108.
15. Vose, J. M.; Bierman, P. J.; Lynch, J. C.; Atkinson, K.; Juttner, C.; Hanania, C. E.; Bociek, G.; Armitage, J. O. Biol Blood Marrow Transplant 2001, 7, 680.
16. Carreras, E.; Saiz, A.; Marin, P.; Martinez, C.; Rovira, M.; Villamor, N.; Aymerich, M.; Lozano, M.; Fernandez-Aviles, F.; Urbano-Izpizua, A.; Montserrat, E.; Graus, F. Haematologica 2003, 88, 306.
17. Gryn, J.; Shadduck, R. K.; Lister, J.; Zeigler, Z. R.; Raymond, J. M. J Hematother Stem Cell Res 2002, 11, 719.
18. Higuchi, A.; Shindo, Y.; Gomei, Y.; Mori, T.; Uyama, T.; Umezawa, A. J Biomat Sci- Polym B 2005, 74, 511.

19. Higuchi, A.; Tsukamoto, Y. J Biomed Mater Res 2004, 71, 470.
20. Yasutake, M.; Sumita, M.; Terashima, S.; Tokushima, Y.; Nitadori, Y.; Takahashi, T. A. Vox Sang 2001, 80, 101.
21. Muller-Steinhardt, M.; Hennig, H.; Kirchner, H.; Schlenke, P. Transfusion 2002, 42, 153.
22. Komai, H.; Naito, Y.; Fujiwara, K.; Takagaki, Y.; Noguchi, Y.; Nishimura, Y. Perfusion 1998, 13, 27.
23. Debelak, J.; Shlomchik, M. J.; Snyder, E. L.; Cooper, D.; Seropian, S.; Mcguirk, J.; Smith, B.; Krause, D. S. Transfusion 2000, 40, 1475.
24. Florian, S.; Sonneck, K.; Hauswirth, A. W.; Krauth, M. T.; Schernthaner, G. H.; Sperr, W. R.; Valent, P. Leuk Lymphoma 2006, 47, 207.
25. Keeney, M.; Chin-Yee, I.; Weir, K.; Popma, J.; Nayar, R.; Sutherland, D. R. Cytometry (Comm Clin Cytom) 1998, 34, 61.
26. Fujimoto, N.; Fujita, S.; Tsuji, T.; Toguchida, J.; Ida, K.; Suginami, H.; Iwata, H. Biomaterials 2007, 28, 4795.
27. Forraz, N.; Pettengell, R.; Mcguckin, C. P. Stem Cells 2004, 22, 100.
28. Wagner, W.; Wein, F.; Roderburg, C.; Saffrich, R.; Faber, A.; Krause, U.; Schubert, M.; Benes, V.; Eckstein, V.; Maul, H.; Ho, A. D. Exp Hematol 2007, 35, 314.
29. Chua, K. N.; Chai, C.; Lee, P. C. Biomaterials 2006, 27, 6043.
30. Krause, D. S.; Fackler, M. J.; Civin, C. I.; May, W. S. Blood 1996, 87, 1.
31. Lanza, F.; Healy, L.; Sutherland, D. R. J Biol Regul Homeost Agents 2001, 15, 1.