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Cell Separation through chemically modified polyurethane membranes

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Abstract

Cell separation from peripheral blood was investigated using surface-modified polyurethane (PU) membranes with different functional groups. Both red blood cells and platelets could pass through unmodified PU and PU-SO_3H membranes, while the red blood cells preferentially passed through PU-$N(C_2H_5)_2$ and PU-NHC_2H_4OH membranes. The permeation ratio of T and B cells was less than 25% for the surface-modified and unmodified PU membranes.

$CD34^+$ cells have been recognized as various kinds of stem cells including hematopoietic and mesenchymal stem cells. The adhesiveness of $CD34^+$ cells on the PU membranes was found to be higher than that of red blood cells, platelets, T cells or B cells. Overall, the adhesiveness of blood cells on the PU membranes increased in the following order: red blood cells $\leq$ platelets < T cells $\leq$ B cells < $CD34^+$ cells. Treatment of PU-COOH membranes with a human albumin solution to detach adhered blood cells, allowed recovery of mainly $CD34^+$ cells in the permeate, while both red blood cells and platelets could be isolated in the permeate using unmodified PU membranes. The PU membranes showed different permeation and recovery ratios of specific cells depending on the functional groups attached to the membranes.

Introduction

Blood cell separation is an important technology for the transplantation of blood cells and hematopoietic stem cells [1]. Purification of $CD34^+$ cells from peripheral blood and umbilical cord blood is reported to reduce contamination of myeloma cells in graft. This leads to improved patient survival and prevents acute or chronic graft-versus-host disease (GVHD), which is due to decreased T cell number [2]. Specific T cell selection (separation), which has ability of GVL (graft-versus-leukemia effect) but does not have ability of GVHD will enable the clinical application of lymphocyte therapy, because the T cells responsible to GVL and GVHD may be different [3].

Blood cell separation is typically carried out by centrifugation, fluorescence activated cell sorting (FACS) [3], magnetic cell selection [4] or membrane filtration. The centrifugal separation of blood cells is a well-known method to separate platelets, leukocytes and red blood cells, but this method does not work well for the separation of cells with similar characteristics, such as T and B cells or $CD34^+$ cells and mononuclear cells. The most highly purified cellular preparations are obtained by FACS in conjunction with a fluorescently-labeled antibody for cell-surface marker. However, this cannot be used for clinical applications because of difficulties with sterility and the excessive time needed to purify sufficient quantities of $CD34^+$ cells.

An alternative to these methods is to use a magnetic cell selection system (e.g., Isolex

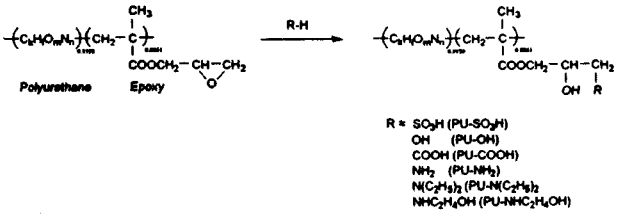
magnetic cell selection system, Baxter healthcare or CliniMACS, Miltenyi Biotec), in which magnetic beads attaching an anti-CD34 monoclonal antibody are mixed with cells from peripheral blood, umbilical cord blood or bone marrow. The magnetic beads attaching anti-CD34 monoclonal antibody are separated by magnetic force to collect the $CD34^+$ cells. Mobilized autologous aphaeresis by magnetic cell selection has been reported to result in a 91.7% purification of $CD34^+$ cells with a 55% recovery [4].

Membrane filtration has also been used for the industrial separation of blood cells. Blood for transplantation is typically passed through membrane filters to eliminate leukocytes, which can help prevent infection by viruses such as HIV and HCV [5,6]. Compared to other cell separation methods, membrane filtration is simple and inexpensive, and it is easy to maintain sterility during the process. Here we report blood cell separation through polyurethane membranes modified with various functional groups, including $-NH_2$, -COOH and $-SO_3H$.

Experimental

Base membranes used for the chemical modification were sponge polyurethane (PU) porous membranes (Ruby Cell S, Toyo Polymer Co., Ltd.) and sponge PU porous membranes containing 0.61% of epoxy group (PU-epoxy), which were plasma-polymerized using glycidyl methacrylate on the sponge PU porous membranes. The average pore size of the PU and PU-epoxy membranes evaluated from Capillary Flow Porometer measurements (Porous Materials Inc.) was 5.2 μm.

Several 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 some chemicals [1]. Six reactants were employed to open the epoxy ring of the PU-epoxy membranes: Na_2SO_4, H_2SO_4, glycine, NH_3, $NH(C_2H_5)_2$ and $NH_2C_2H_4OH$. The anticipated product by the ring-opening reaction of epoxy group is shown in Scheme 1. The resultant membranes were referred to as $PU\text{-}SO_3H$, PU-OH, PU-COOH, $PU\text{-}NH_2$, $PU\text{-}N(C_2H_5)_2$ and $PU\text{-}NHC_2H_4OH$ membranes.



Scheme 1

A schematic of the blood filtration apparatus is shown in Fig. 1. A sample of 6 ml of human fresh blood (man, 22 years old) was filtered through membranes attached inside the membrane holder at the filtration rate of 1 ml/min. 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.). The permeation ratio is defined as

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

After the blood filtration, the membranes were upside down inside the membrane holder, and 0.5-

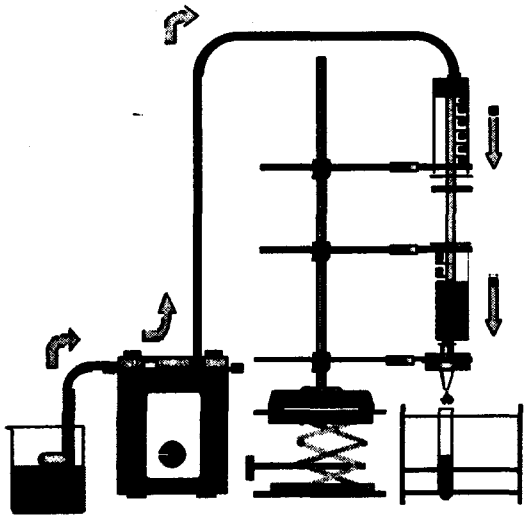


Fig. 1 Permeation apparatus.

wt% human serum albumin (HSA) solution was permeated through the membranes using the same membranes and the apparatus at filtration speed of 1 ml/min to remove the attached cells on the membranes and to collect them in the HSA solution.

The recovery ratio is defined as

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

Where N_r is the number of cells in the permeate solution after the permeation of HSA solution. The filtration experiments were performed at 25±0.5 °C.

The number of red blood cells and platelets in the feed (peripheral blood) and permeate solutions was analyzed from surface markers of glycophorin A for red blood cells and CD41 for platelets, respectively. The number of T cells and B cells in the feed (peripheral blood) and permeate solutions was analyzed from surface markers of CD3 and CD19, respectively. The number of hematopoietic stem cell was analyzed by $CD34^+$ cells followed by ISHAGE (International Society of Hemathotherapy and Graft Engineering) guidelines using Stem-Kit™ (Beckman Coulter Co.) and the flow cytometry [1].

Results and Discussion

The numbers of red blood cells and platelets in the permeate were analyzed by flow cytometry after passing blood through the surface-modified and unmodified PU membranes. Figure 2 shows the permeation ratio of red blood cells and platelets through the membranes. Both red blood cells and platelets could pass through unmodified PU and PU-SO_3H membranes, while red blood cells preferentially passed through PU-$N(C_2H_5)_2$ and PU-PU-NHC_2H_4OH membranes. PU-COOH, PU-OH and PU-NH_2 membranes showed relatively lower permeation ratios of red blood cells and platelets compared to unmodified PU membranes.

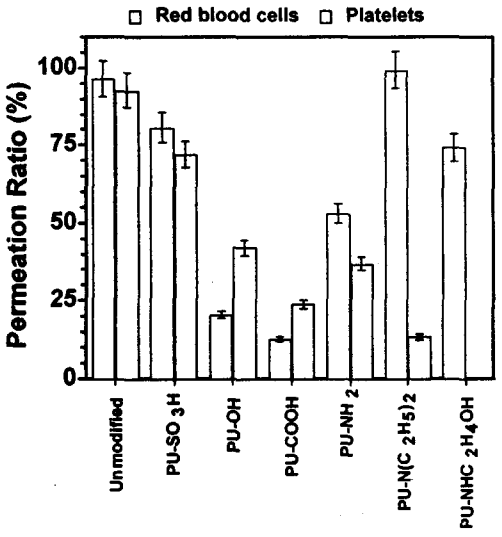


Fig. 2 Permeation ratio of red blood cells and platelets through unmodified and surface-modified PU membranes.

HSA solution was passed through the PU membranes following permeation of the blood. The recovery ratio was found to be less than 25% for the surface-modified and unmodified PU membranes. This low recovery may have been due to the fact that the most of the red blood cells and platelets had already passed through the membranes when the blood was passed through the membranes.

The numbers of T and B cells in the permeate solution were analyzed by flow cytometry after passing blood through the surface-modified and unmodified PU membranes. Figure 3 shows the permeation ratio of T and B cells. The permeation ratio was less than 25% for the surface-modified and unmodified PU membranes. In fact, the PU-

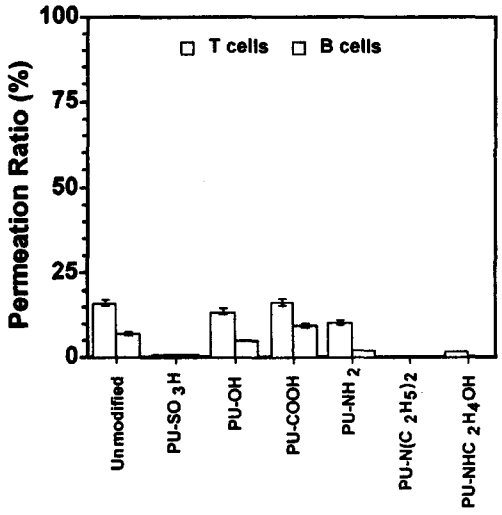


Fig. 3 Permeation ratio of T and B cells through unmodified and surface-modified PU membranes.

SO_3H, PU-$N(C_2H_5)_2$ and PU-NHC_2H_4OH membranes showed a permeation ratio of less than 5%. This indicates that the T and B cells adhered stronger to these membranes than red blood cells and platelets. Using surface-modified and unmodified PU membranes, the permeation ratio is higher for T cells than for B cells. Therefore, B cells adhered to the membranes stronger than T cells.

A HSA solution was passed through the PU membranes after permeation of blood to remove some of the bound cells. The recovery ratio of T and B cells was the highest after permeation of HSA through the PU-SO_3H. This indicates that T and B cells attached weakly to the PU-SO_3H membranes, and that the cells easily detach from the membrane surface when HSA solution passes through the membranes. We also found that the recovery ratio of T and B cells was relatively high after passing HSA solution through the PU-COOH and PU-NHC_2H_4OH membranes.

The recovery ratio of T cells was found to be higher than that of B cells when using the surface-modified and unmodified PU membranes. These results also indicate that the B cells adhere to the membranes more strongly than T cells.

$CD34^+$ cells have been recognized as various kinds of stem cells, including hematopoietic and mesenchymal stem cells. We examined the permeation of hematopoietic stem cells through the surface-modified and unmodified PU membranes using a Stem-Kit™ in conjunction with a $CD34^+$ cell surface marker. Figure 4 shows the permeation ratio of $CD34^+$ cells through the surface-modified and unmodified PU membranes. The permeation ratio was less than 5% for the surface-modified and unmodified PU membranes. This indicates that the $CD34^+$ cells adhere to the membranes stronger than red blood cells, platelets, T cells and B cells. The adhesion of blood cells to the PU membranes appeared to increase in the following order: red blood cells ≤ platelets < T cells ≤ B cells < $CD34^+$ cells.

HSA solution was passed through the PU membranes after permeation of blood. Figure 4 also shows the recovery ratio of $CD34^+$ cells. The recovery ratio of $CD34^+$ cells was the highest after permeation of HSA solution through PU-COOH membranes. This result was unexpected because the PU-SO_3H membranes allowed the highest recovery of T and B cells and because the PU-SO_3H membranes were expected to give the highest recovery of $CD34^+$ cells before HSA permeation. Therefore, it appears that not only the pore structure and surface charge but also the specific functional group on the membrane surface can regulate the attachment and detachment of specific cells.

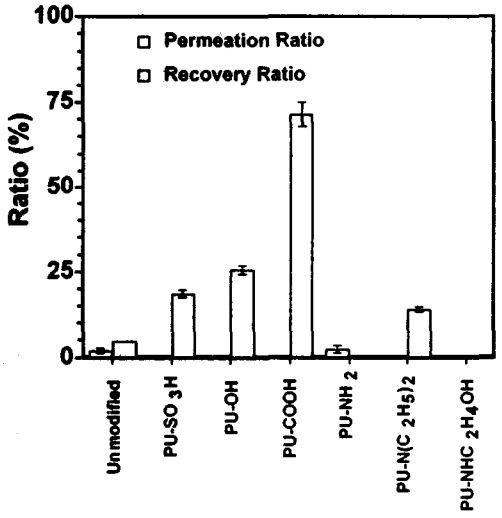


Fig. 4 Permeation and recovery ratio of $CD34^+$ cells through unmodified and surface-modified PU membranes.

1. A. Higuchi et al., J. Biomed. Mater. Res., 68A (2004) 34.
2. R.A. Preti et al., Cytotherapy 3 (2001) 85.
3. Y. Sanada, In: M. Harada et al., editors. New trends in hematopoietic stem cell transplantation. Tokyo: Nanoudo, 1998. p1-7.
4. J. Gryn, J. Hematother. Stem Cell Res., 11 (2002) 719.
5. H. Komai et al., Perfusion, 13 (1998) 27. 6. M. Muller-Steinhardt et al., Transfusion, 42 (2002) 153.