Bioinert Surface of Pluronic-Immobilized Flask for Preservation of Hematopoietic Stem Cells

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The bioinert materials on which cells do not proliferate, differentiate, nor de-differentiate should be useful for the culture and preservation of stem cells. The Pluronic F127, a triblock copolymer of ethylene oxide, and propylene oxide was activated using carbonyldiimidazole (CDI), and CDI-activated Pluronic was subsequently immobilized on the surface of a lysine-coated polystyrene tissue culture flask. The morphology of fibroblasts (L929 cells) on the Pluronic-immobilized flask was spherical, and did not show spreading behavior. This observation indicates that L929 cells on the Pluronic-immobilized flask were cultured in a bioinert environment. The expression ratio of surface markers on hematopoietic stem cells (CD34 and CD133) cultured in the Pluronic-immobilized flask was significantly higher than that in polystyrene tissue culture flask and commercially available bioinert flask (i.e., low cell binding cultureware). This is caused by the existence of hydrophilic segments of Pluronic F127 on the Pluronic-immobilized flask.

Introduction

Cell culture materials in which cells proliferate and differentiate have been investigated by many researchers[1−7] because most cells (except blood cells) are anchorage-dependent. A key direction to research the interaction between cells and materials in plastic and reconstructive surgery is how the stem cells attach to the scaffolds[5−7] or injectable materials for transplantation into tissue defects in patients. Once the cells are attached to the surface of the material, intracellular signals regulating proliferation and differentiation of cells are generated via interaction between specific receptors and cell signaling molecules adsorbed or expressed on the materials. However, the surfaces of bioinert materials on which cells do not proliferate, differentiate, nor de-differentiate have not yet been studied extensively. These materials should be useful for the culture and/or preservation of embryonic stem (ES) cells[8,9] and various other kinds of stem cells, including hematopoietic stem cells.[10,11]

In our previous investigation,[12] we examined plasma protein adsorption and platelet adhesion to polysulfone membranes coated with Pluronic of varying poly(ethylene oxide) (PEO) block lengths. The triblock copolymer Pluronic, composed of PEO−polypropylene oxide (PPO)−PEO triblocks, exhibits amphiphilic properties and undergoes self-assembly into micelles, as well as forming a lyotropic liquid crystal gel phase, due to its hydrophilic PEO and hydrophobic PPO blocks.[13−16] Results suggested that the bioinert property of PEO segments in Pluronic, attributed to their high flexibility in aqueous media, suppressed the adsorption of plasma proteins and platelets.[16]

The cell interaction and adhesion to Pluronic surfaces were also studied by several researchers.[17,18] Liu et al. used Pluronic F108 to create micropatterned nonadhesive domains on the surfaces made of tissue culture polystyrene, methylated glass, and polylactic-*co*-glycolic acid.[17] The effectiveness of the Pluronic in inhibiting cell adhesion was found even in the presence of collagen I.[17] Chandaroy et al. prepared liposomes associated with Pluronic F127 that inhibits liposome−cell adhesion.[18] Attachment of the Pluronic containing liposomes to CHO cells was inhibited at temperatures above the critical micellar temperature, but not at temperatures below critical micellar temperature.[18] This observation indicated that temperature-sensitive control of liposome−cell adhesion was achieved by using temperature sensitive properties of Pluronic F127.

Cell cultures of fibroblasts on Pluronic gels were also examined in our previous investigation.[2] However, cell culturing was successful for only 48 h, because the Pluronic gels were too hydrophilic and tended to dissolve in the culture medium. Therefore, in this study we developed the Pluronic-immobilized tissue culture flask, with covalent bonding between Pluronic and the flask. Here we show that, compared to three commercially available types of tissue culture flask, hematopoietic stem cells expressing cell surface markers, CD34 and CD133, in umbilical cord blood are preserved for an extended time in the Pluronic-immobilized tissue culture flask at 4 °C. We propose that the existence of hydrophilic segments of Pluronic F127 on the tissue culture flask induces bioinert storage of hematopoietic stem cells in umbilical cord blood.

Experimental Section

Materials. Pluronic F127 (MW = 12 000 g mol^{-1}, $PEO_{99}-PPO_{65}-PPO_{99}$) was supplied by Asahi Electric Industry Co. Ltd. *N*,*N*′-Carbonyldiimidazole (CDI) was from Tokyo Kasei Co. and was used as received. The 24-well tissue culture flask coated with poly-L-lysine (4820-040, polylysine-coated flask, PLL) and 24-well polystyrene tissue culture flask (3820-024, PSt tissue culture flask) were purchased from Asahi Techno Glass. HydroCell flask and RepCell flask were purchased

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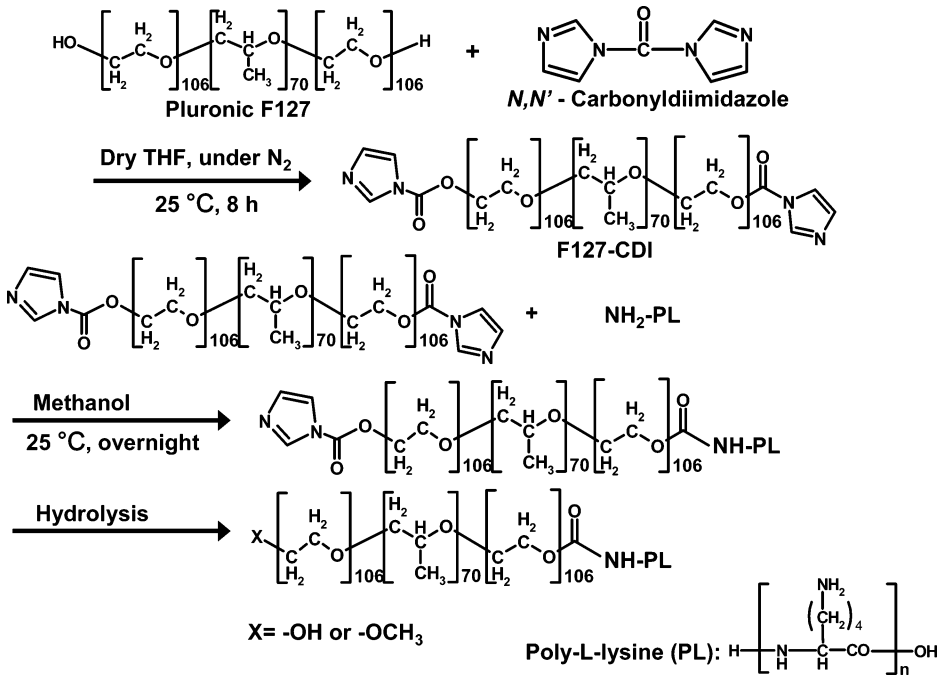

Figure 1. Reaction scheme of CDI-activated Pluronic F127 from Pluronic F127 and immobilization of CDI-activated Pluronic F127 on the polylysine-coated flask.

from CellSeed Inc. Micro BCA protein assay reagent kit (Pierce Biotechnology Inc.) was used to measure the coating amount of poly-L-lysine on polylysine-coated flask. Tetrahydrofuran (THF, Wako Pure Chemicals, Ltd.) was dried in sodium and redistilled before use. Anti-CD34 antibody (IgG_1, κ) conjugated with fluorescein isothiocyanate (FITC) (555821, BD Biosciences Pharmingen), anti-CD133 antibody (IgG2b) conjugated with phycoerythrin (PE) (130-090-853, Miltenyi Biotec GmbH), mouse antibody (IgG_1, κ) conjugated with FITC (555748, BD Biosciences Pharmingen), and mouse antibody (IgG_1, κ) conjugated with PE (555749, BD Biosciences Pharmingen) were used as received. FcR blocking reagent (130-059-901; Miltenyi Biotec GmbH), propidium iodide solution (51-66211E, BD Biosciences Pharmingen), and flow-count beads solution (7547053; Beckman Coulter Co.) were also used as received. Other chemicals were of 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 CDI-Activated Pluronic F127. CDI-activated Pluronic F127 was prepared from the procedure similar to CDI-activated Pluronic F68 reported by Lu et al.[19] The reaction scheme is shown in Figure 1. Pluronic F127 was purified by dissolving in acetone and precipitating into an excess amount of cooled hexane, and it was dried under vacuum at room temperature for 12 h. The purified Pluronic F127 (5.04 g, 0.42 mmol) was dissolved in dry THF (60 mL) and added dropwise to an excess amount of CDI (3.24 g, 20 mmol) in THF (60 mL) at room temperature during a 6 h period under nitrogen atmosphere. After the addition, the mixture was kept stirring for an additional 2 h. The solution was concentrated to a small volume under vacuum and poured into 600 mL of ethyl ether. The precipitate was collected by filtration. This process was repeated three times to remove unreacted CDI. The CDI-activated Pluronic F127 was obtained as white powder after drying under vacuum at room temperature for 12 h (yield 4.6 g, 89%).

Preparation of Pluronic-Immobilized Flask. Two microliters of CDI-activated Pluronic F127 in methanol (0−10 mg/mL) was inserted into the polylysine-coated flask. The flask was incubated for 24 h at 25 °C under a shaking incubator. The Pluronic F127-immobilized tissue culture flask (Pluronic-immobilized flask) was rinsed with methanol three times and with ultrapure water five times, subsequently. Finally the Pluronic-immobilized flask was dried under vacuum for 12 h. PL-*X* at $X = 0.1$, 0.25, 0.5, and 10 indicates the Pluronic-immobilized flask prepared on the condition of the concentration of CDI-activated Pluronic F127 = 0.1, 0.25, 0.5, and 10 mg/mL, respectively.

Characterization of Pluronic-Immobilized Flask. The coating amount of poly-L-lysine on the polylysine-coated flask was estimated from micro BCA protein assay.[12] Briefly, 0.1 mL of poly-L-lysine solution (0−100 ppm, pH 7.2, phosphate buffer solution containing poly-L-lysine) and 0.1 mL of BCA working reagent were mixed well (standard solution). A total of 0.1 mL of phosphate buffer solution (pH 7.2) and 0.1 mL of BCA working reagent were injected into the polylysine-coated flask and mixed well (sample solution). Both standard and sample solutions were incubated at 60 °C for 60 min. After incubation, the standard and sample solutions were cooled to the room temperature. The absorbance of 200 μL of the standard and sample solutions was measured at 562 nm using a microplate reader (Model 550, Bio-Rad Laboratories). The calibration curve was obtained from the absorbance of the standard solution. The amount of poly-L-lysine on the polylysine-coated flask ($C_{polylysine}$) was measured from the following equation

$$C_{polylysine} = 0.1 \times C_{solution}/A \quad (1)$$

where $C_{solution}$ is the concentration of poly-L-lysine in the sample solution obtained from the calibration curve. *A* is the surface area where the sample solution contacted on the polylysine-immobilized flask.

The water contactangles of the Pluronic-immobilized flask and poly-L-lysine-coated flask were measured at 25 °C and 85% relative humidity by the sessile drop method using ultrapure water,[1,12] after the surface of the flasks was cut into the circle having the diameter of 1 cm. The water contact angles were monitored and recorded with a CCD camera (DCR-PC100, Sony Corporation). At least four readings ($n = 4$) were taken for 5 min after placing water droplets (20 μL) on different plates of the flasks, and the values were averaged.

Atomic analysis of the surface of Pluronic-immobilized flask was performed using XPS (ESCA-3400, Kratos Analytical Ltd).

Cell Cultivation of L929 Cells. L929 cells (Dainippon Seiyaku Co., Ltd.), derived from a mouse connective tissue fibroblast, were maintained in RPMI 1640 media (JRH Bioscience, Lenexa, KS) supplemented with 25 mg/L streptomycin sulfate, 3.5 mg/L benzyl-penicillin potassium, and 10% fetal bovine serum (FBS, JRH Bioscience, Lenexa, KS) in 5% CO_2 atmosphere at 37 °C.[20] The cell lines were used between passage numbers 10 to 20 in the following

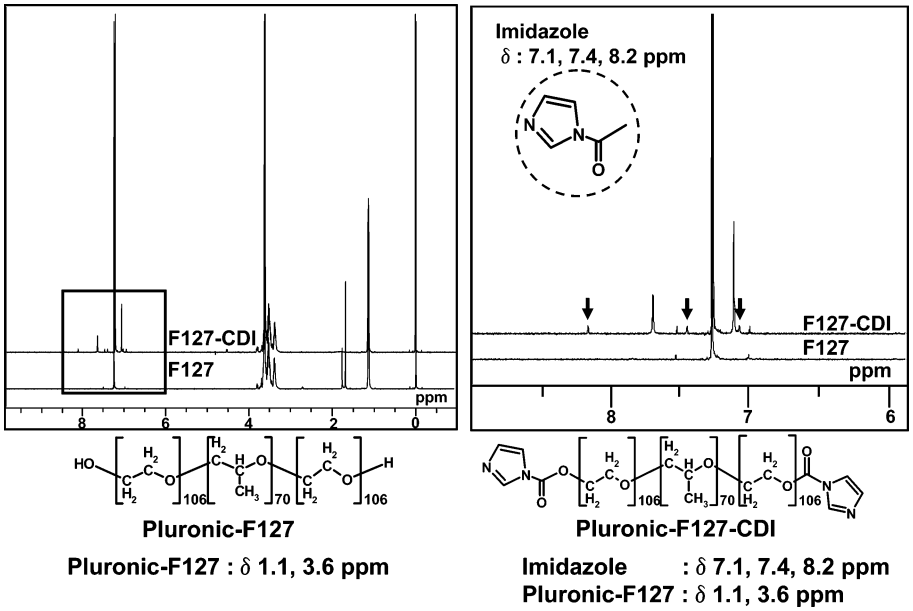

Figure 2. ^{1}H NMR spectra of CDI-activated Pluronic F127. The solvent used was $CDCl_3$. The arrows indicate the protons on the imidazole moiety.

experiments. L929 cells in suspension (cell densities 5×10^4/cm^2 and 2 mL) were injected into the Pluronic-immobilized flasks and tissue culture flasks, and they were incubated in the CO_2 incubator in 5% CO_2 atmosphere at 37 °C. The numbers of cells were counted using an inverted microscope (Diaphoto TMD300, Nikon Co.) equipped with a CCD video camera, ARGUS 20 (Hamamatsu Photonics K. K.), digital camera (Camedea, Olympus Co.), and a temperature-regulated box.[20−22] The cell number was obtained from the average of four different experiments on each well using four independent wells prepared under the same conditions (total $n = 16$). The dynamic morphology of the cells was also recorded using the inverted microscope equipped with the CCD video camera and temperature-regulated box.

Storage of Umbilical Cord Blood Cells. Umbilical cord blood was obtained after deliveries with informed consent. A sample of 35 mL of human umbilical cord blood was collected using five vacuum tubes (7 mL, Venoject II, Terumo, Co.) containing 10.5 mg of EDTA 2Na. Human umbilical cord blood was injected into the Pluronic-immobilized flask, HydroCell flask, RepCell flask, and polystyrene tissue culture flask, and it was stored in a refrigerator for 7 days at 4 °C. One milliliter of the umbilical cord blood was sampled every day and analyzed using flow cytometry (Coulter EPICS XL; Beckman-Coulter Co.) for measurement of surface markers of hematopoietic stem cells (i.e., CD34 and CD133), after dyeing of cells with anti-CD34 antibody and anti-CD133 antibody by a conventional method reported previously.[21,23] The same procedures for the surface marker analysis, except with the use of mouse immunoglobulins (IgG_1, κ) conjugated with FITC and PE instead of anti-CD34 antibody conjugated with FITC and anti-CD133 antibody conjugated with PE, respectively, were also performed to check the nonspecific adsorption of anti-CD34 antibody and anti-CD133 antibody to the cells.

Results and Discussion

Characterization of Pluronic-Immobilized Flask. Figure 1 illustrates the synthesis of CDI-activated Pluronic F127. The ^{1}H NMR spectra of CDI-activated Pluronic F127 confirmed its structure, as shown in Figure 2. The peaks at δ 8.2, δ 7.4, and δ 7.1 ppm were attributed to the protons on the imidazole moiety, whereas those at δ1.1 and δ3.6 ppm correspond to the protons of Pluronic F127. Table 1 shows the elemental atomic concentration of CDI-activated Pluronic F127 calculated and that experimentally obtained from the elemental atomic analysis.

Table 1. Elemental Atomic Concentration of CDI-Activated Pluronic F127

	carbon (atomic %)	hydrogen (atomic %)	nitrogen (atomic %)
experimental	58.8	9.70	0.51
calculated	60.5	10.0	0.44

The experimentally obtained atomic concentrations of carbon, hydrogen, and nitrogen in CDI-activated Pluronic F127 were found to be nearly equal to the calculated concentrations. Therefore, CDI-activated Pluronic F127 was synthesized with approximately 100% conversion based on the atomic analysis as well as the peak area comparison between the imidazole proton (peak areas at δ 8.2, δ 7.4, and δ 7.1 ppm) and the Pluronic F127 proton (peak areas at δ 1.1 and δ 3.6 ppm).

The Pluronic-immobilized flask was prepared by reaction of CDI-activated Pluronic F127 on the surface of a 24-well tissue culture flask coated with poly-L-lysine, as shown in Figure 1. The end opposite to the binding end of Pluronic F127 chains, attached to the Pluronic-immobilized flask, is expected to be cleaved by hydrolysis of the CDI-activated Pluronic F127 imidazole ring to −OH and/or $-OCH_3$, as shown in Figure 1. There is a possibility that some of the CDI-activated Pluronic F127 has only monofunctional CDI. However, monofunctional CDI-activated Pluronic F127 is sufficient to react with poly-L-lysine on the polylysine-coated flask. The end opposite to the binding end of Pluronic F127 chains, attached to the Pluronic-immobilized flask, remains as unreacted −OH of the monofunctional CDI-activated Pluronic F127 in this case.

Because the amount of poly-L-lysine in aqueous poly-L-lysine solution could be measured from a calibration curve by the micro BCA protein assay using a known concentration of aqueous poly-L-lysine solution, the amount of poly-L-lysine on the polylysine-coated flask used in this investigation ($C_{Polylysine}$) was estimated to be 11.5 μg/cm^2 using the calibration curve of aqueous poly-L-lysine solution from the micro BCA protein assay.

XPS analysis of the polylysine-coated flask and Pluronic-immobilized flask was performed. Table 2 shows the atomic mole fraction ($O1_S/C1_S$ and $N1_S/C1_S$) of the polylysine-coated

Table 2. Atomic Concentration Ratio of Polylysine-Coated Flask and Pluronic-Immobilized Flask Analyzed by XPS, Reaction Ratio, Surface Concentration of Pluronic ($C_{Pluronic}$), and Water Contact Angles on the Flask

sample	reaction concn of Pluronic (mg/mL)	concentrati on ratio O/C	concentrati on ratio N/C	reaction ratio (%)	$C_{Pluronic}$ (nmol/cm^2)	water contact angle (deg)
PLL	0	1.5×10^{-1}	3.2×10^{-2}	0	0	63.5
PL-0.1	0.10	1.5×10^{-1}	3.1×10^{-2}	2.0	1.8	51.5
PL-0.25	0.25	1.5×10^{-1}	3.1×10^{-2}	3.2	2.8	44.0
PL-0.5	0.50	1.6×10^{-1}	3.0×10^{-2}	4.7	4.2	40.0
PL-10	10.0	2.6×10^{-1}	2.0×10^{-2}	39.	35.	34.0

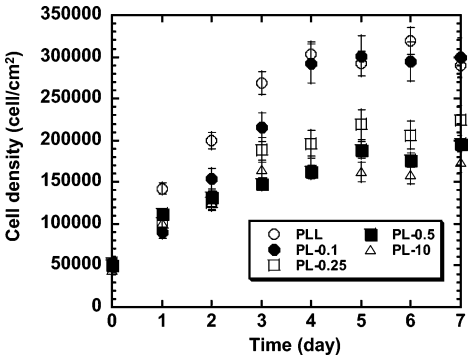

Figure 3. Growth curves of L929 cells cultured on polylysine-coated flask (PLL) and Pluronic-immobilized flask (PL-0.1, PL-0.25, PL-0.5, and PL-10). Data are expressed as means ± SD of four independent measurements.

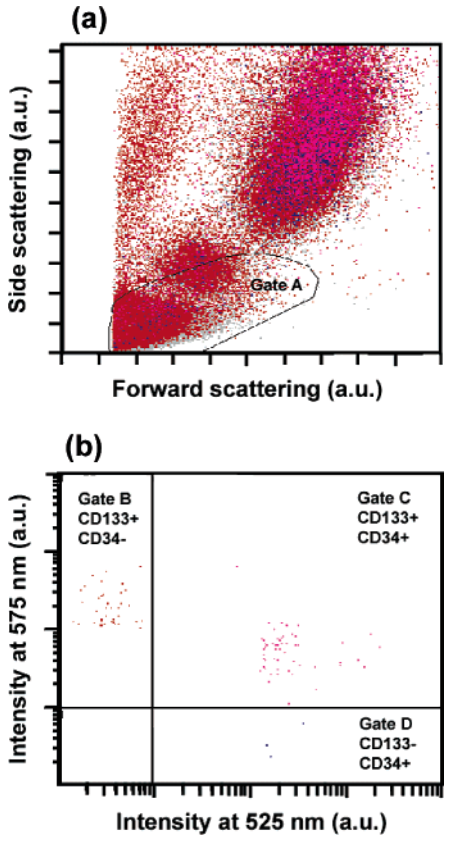

Figure 4. Flow cytometric scattergrams of umbilical cord blood cells at the intensity of side light scattering and intensity of forward light scattering (a) and at the fluorescent intensities of 575 nm from anti-CD133 antibody conjugated with PE and 525 nm from anti-CD34 antibody conjugated with FITC (b).

flask and Pluronic-immobilized flask. The mole fraction of oxygen to carbon for poly-L-lysine and polystyrene were calculated to be 0.167 and 0, respectively, whereas those of nitrogen to carbon for poly-L-lysine and polystyrene were 0.334 and 0, respectively. XPS measurements revealed that from the mole fraction of the tissue culture flask coated with poly-L-lysine shown in Table 1, the mole fraction of the existence of poly-L-lysine on the surface of the tissue culture flask coated with poly-L-lysine was estimated to be 9.5% from the calculation of 3.2/0.334. The reaction ratio of CDI-activated Pluronic F127 to the amino group of poly-L-lysine coated on the tissue culture flask (R) was also calculated from XPS analysis of Pluronic-immobilized flask prepared with different concentrations (0−10 mg/mL) of CDI-activated Pluronic F127 solution. These values were obtained using the mole fraction of oxygen to carbon and nitrogen to carbon on the surface, where CDI-activated Pluronic F127 was perfectly reacted with all of the amino groups of poly-L-lysine coated on the tissue culture flask. The obtained values were calculated to be 0.434 and 8.25×10^{-4} for oxygen to carbon and nitrogen to carbon, respectively. The reaction ratio was calculated from the mole fraction of oxygen to nitrogen ($f_{O/C}$) and that of nitrogen to carbon ($f_{N/C}$) on the Pluronic-immobilized flask as follows:

$$\text{Reaction ratio (\%)} = (f_{O/C} - 1.5 \times 10^{-1}) \times 100/(0.434 - 1.5 \times 10^{-1}) \quad (2)$$

$$\text{Reaction ratio (\%)} = 100 - (f_{N/C} - 8.25 \times 10^{-4}) \times 100/(3.2 \times 10^{-2} - 8.25 \times 10^{-4}) \quad (3)$$

The reaction ratio calculated from eqs 2 and 3 was averaged and is summarized in Table 2. The reaction ratio was found to increase with an increase in concentration of CDI-activated Pluronic F127 (C_{CDI}) in the reaction. The surface concentration of Pluronic F127 on the Pluronic-immobilized flask ($C_{Pluronic}$) can be calculated from the following equation

$$C_{Pluronic} = C_{Polylysine} \times R/MW_{lysine} \quad (4)$$

where MW_{lysine} is the unit molecular weight of poly-L-lysine (128.2 Da). $C_{Pluronic}$ can be controlled by the concentration of the reaction solution (C_{CDI}), because the reaction ratio is regulated by the concentration of the reaction solution (C_{CDI}). $C_{Pluronic}$ was found to be 0−35 nmol/cm^2 in the current work.

The water contact angle of the Pluronic-immobilized flask and polylysine-coated flask was investigated using the sessile drop method at 25 °C, and the results are also summarized in Table 2. The hydrophilicity of the Pluronic-immobilized flask increased with an increase in the surface concentration of Pluronic F127 ($C_{Pluronic}$).

Cell Culture on Pluronic-Immobilized Flask. The cell growth and morphology of L929 cells cultured on the polylysine-coated flasks and Pluronic-immobilized flasks with different Pluronic concentrations on the surface were examined by phase contrast microscopy. Results were used as an index of cell behavior and function. The growth kinetics of L929 cells are shown in Figure 3. The cells on the Pluronic-immobilized flask increased up to 4−5 days, showing approximately constant

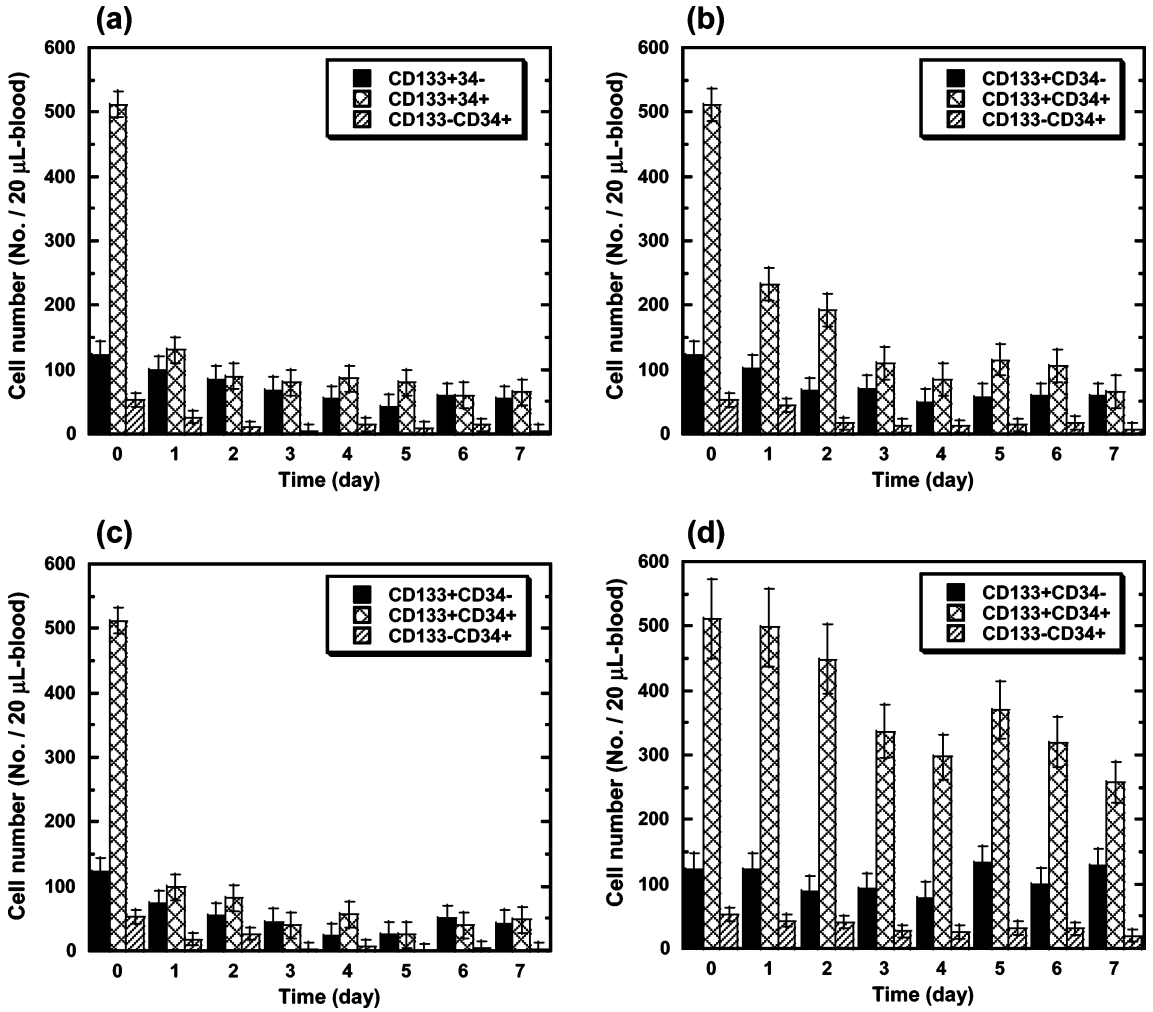

Figure 5. Time dependence of expression ratio of $CD133^{+}CD34^{-}$, $CD133^{+}CD34^{+}$, and $CD133^{-}CD34^{+}$ of umbilical cord blood cells, evaluated from flow cytometry. The cells were incubated on a polystyrene tissue culture flask (a), a RepCell flask (b), a HydroCell flask (c), and a Pluronic-immobilized flask (d, PL-10) for 7 days at 4 °C. Data are expressed as means ± SD of four independent measurements.

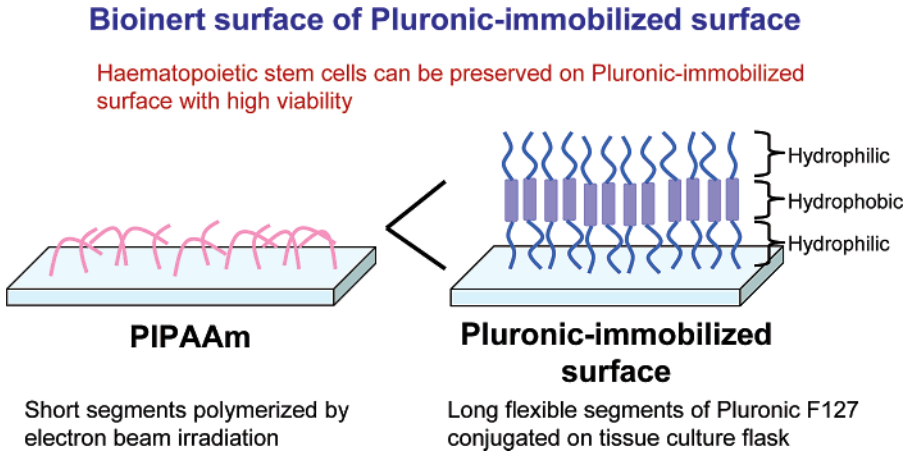

Figure 6. Schematic representation of the surface of RepCell flask and Pluronic-immobilized flask.

cell density after 5 days of incubation at any surface concentration of Pluronic F127 in this investigation. The cell density at 5 days of incubation on the Pluronic-immobilized flask at $C_{Pluronic} \geq 2.5$ nmol/cm^2 was found to be lower than that at $C_{Pluronic} < 2.5$ nmol/cm^2. The growth rate of cells on the Pluronic-immobilized flask at $C_{Pluronic} \geq 2.5$ nmol/cm^2 was also observed to be lower than those at $C_{Pluronic} < 2.5$ nmol/cm^2. This is a result of higher hydrophilicity on the surface of the Pluronic-immobilized flask at $C_{Pluronic} \geq 2.5$ nmol/cm^2 relative to that at $C_{Pluronic} < 2.5$ nmol/cm^2. This is thought to be because the extremely hydrophilic surface originated from Pluronic F127 segments on the surface is unfavorable for the cell culture.[24]

The dynamic morphologies of L929 cells on a Pluronic-immobilized flask (PL-10, $C_{Pluronic} = 35$ nmol/cm^2) and a polystyrene tissue culture flask were recorded for 2−14 h after inoculation of L929 cells, and they are shown in Appendix 1 and 2 in the Supporting Information, respectively. The L929 cells on the Pluronic-immobilized flask had a round shape and

were expected to be undifferentiated, indicating that the cells were weakly attached to the flask surface. In contrast, several cells showing filopodia and a flattened morphology were observed on the polystyrene tissue culture flask. The hydrophilic surface of the Pluronic-immobilized flask is unfavorable for cell proliferation, with the result that cells are in the undifferentiated condition.

Surface Marker Expression of Hematopoietic Stem Cells. As cell culturing on the Pluronic-immobilized flask can be performed under very inert conditions, we decided to examine the possibility of preserving hematopoietic stem cells on the flask. Umbilical cord blood was stored at 4 °C in the Pluronic-immobilized flask as well as a conventional polystyrene tissue culture flask and commercially available bioinert flasks (HydroCell and RepCell), and flow cytometric analysis of surface markers was performed on hematopoietic stem cells after cultivation. The surface of the RepCell flask is reported to be modified with poly(*N*-isopropylacrylamide) (PIPAAm) by electron-beam polymerization,[25] while the surface of HydroCell flask is composed of a bioinert material. Human umbilical cord blood was used for these experiments, because it is known to express several surface markers of hematopoietic stem cells, including CD34 and CD133.[26,27]

Figure 4a,b shows the flow cytometric analysis of the umbilical cord blood cells. Following selection of viable cells by propidium iodide staining and selection of cells at the appropriate size, observing forward light scattering and side light scattering (gate A) as shown in Figure 4a, expression of the surface antigens of $CD133^+CD34^-$ (gate B in Figure 4b), $CD133^+CD34^+$ (gate C in Figure 4b), and $CD133^-CD34^+$ (gate D in Figure 4b) was determined. Therefore, the cells analyzed in gate A in Figure 4a were evaluated using the protocol employed in Figure 4b. When class-matched control antibodies conjugated with FITC and PE were used in the flow cytometric analysis of umbilical cord blood, the cell numbers corresponding to gate B, gate C, and gate D were found to be less than 5, 10, and 3 cells/20 μL, respectively. Therefore, the protocols analyzed in this investigation avoid cell-counting the nonspecific adsorption of anti-CD34 and anti-CD133 antibodies to the umbilical cord blood cells.

Figure 5a−d shows the time dependence of the cell numbers of expressed $CD133^+CD34^-$, $CD133^+CD34^+$, and $CD133^-CD34^+$ in umbilical cord blood cultivated on the polystyrene tissue culture plates (Figure 5a), RepCell flasks (Figure 5b), HydroCell flasks (Figure 5c), and Pluronic-immobilized flasks (PL-10, Figure 5d) for 7 days at 4 °C, evaluated by flow cytometry. The number of cells expressing $CD133^+CD34^-$, $CD133^+CD34^+$, and $CD133^-CD34^+$ in umbilical cord blood cultivated on the polystyrene tissue culture flask decreased significantly after 1 day of cultivation. The numbers of cells expressing $CD133^+CD34^-$ and $CD133^+CD34^+$ were less than 150 cells/20 μL and the number expressing $CD133^-CD34^+$ was less than 30 cells/20 μL after 1 day of cultivation on the polystyrene tissue culture flask (Figure 5a) or HydroCell flask (Figure 5c). The RepCell flask (Figure 5b) showed slightly better results for expression of hematopoietic stem cell markers: after 3 days of cultivation, the numbers of cells expressing $CD133^+CD34^-$ and $CD133^+CD34^+$ were less than 120 cells/20 μL and the number expressing $CD133^-CD34^+$ was less than 30 cells/20 μL (Figure 5b). In contrast, the number of cells expressing $CD133^+CD34^+$ in umbilical cord blood on the Pluronic-immobilized flask (Figure 5d) was more than 250 cells/20 μL after 7 days, a result four times higher than those obtained using the normal polystyrene tissue culture flask, flask covered with poly(*N*-isopropylacrylamide) (RepCell), or HydroCell^R flask. Using the Pluronic-immobilized flask, for most typical hematopoietic stem cells ($CD133^+CD34^+$ cells),[28,29] 50% of the original number of umbilical cord blood cells were preserved after 7 days, while only 20% of the initial numbers were preserved on the polystyrene tissue culture flask, HydroCell flask, or RepCell flask after 7 days.

Relatively primitive hematopoietic stem cells ($CD133^+CD34^-$ cells)[28,29] in umbilical cord blood were also preserved on the Pluronic-immobilized flask more effectively than on a polystyrene tissue culture flask, HydroCell flask, or RepCell flask for 7 days. The number of $CD133^+CD34^-$ cells in umbilical cord blood on the Pluronic-immobilized flask was twice as high as the number preserved on the polystyrene tissue culture flask, HydroCell flask, or RepCell flask after 7 days.

Conclusions

Hematopoietic stem cells were extensively preserved in the Pluronic-immobilized flask under bioinert conditions, using umbilical cord blood at 4 °C in this study. This may be due to the flexible and hydrophilic segments of Pluronic F127 on the tissue culture flask, which induce bioinert preservation of hematopoietic stem cells. It was found that hematopoietic stem cells could not be preserved for long on the commercially available bioinert flask (HydroCell). The data shown in Figure 5c may represent the results for the HydroCell flask after detachment of the bioinert coating materials. Hematopoietic stem cells are preserved on the RepCell flask slightly better than on the polystyrene tissue culture flask or the HydroCell flask, but worse than on the Pluronic-immobilized flasks. The RepCell flask is prepared by polymerization of *N*-isopropylacrylamide after electron-beam irradiation of the tissue culture flask;[25] therefore, the segment length of poly(*N*-isopropylacrylamide) should be shorter than that of Pluronic F127. Furthermore, Pluronic F127 is more hydrophilic than poly(*N*-isopropylacrylamide). Pluronic consists of three block regions, with a hydrophilic block−hydrophobic block−hydrophilic block arrangement. Pluronic segments conjugated on the tissue culture flask probably generate a brush-like conformation due to the interaction between adjacent hydrophobic areas (PPO segments) by self-organization in aqueous and blood solutions (Figure 6). It is concluded that the flexible and hydrophilic segments of Pluronic F127 conjugated on the flask surface are the reason for the effective preservation of hematopoietic stem cells in the Pluronic-immobilized flask.

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Supporting Information Available. The dynamic morphologies of L929 cells on a Pluronic-immobilized flask (PL-10, $C_{Pluronic} = 35$ nmol/cm^2) [Appendix 1] and on a polystyrene tissue culture flask [Appendix 2], each recorded for 2−14 h after inoculation of L929 cells. This material is available free of charge via the Internet at http://pubs.acs.org.

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