Bio-inert Surface of Pluronic-immobilized Flask for Preservation of Hematopoietic Stem Cells

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Introduction

The bio-inert materials on which cells do not proliferate, differentiate nor de-differentiate should be useful for the culture and preservation of stem cells. In our previous investigation,[1] we examined plasma protein adsorption and platelet adhesion to polysulfone membranes coated with Pluronic of varying PEO block lengths. Results suggested that the bio-inert property of PEO segments in Pluronic, composed of polyethylene oxide (PEO)-polypropylene oxide (PPO)-PEO triblocks, suppressed the adsorption of plasma proteins and platelets. Cell cultures of fibroblasts on Pluronic gels were examined in our previous investigation.[2] However, cell culturing was successful for only 48 hrs, 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 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 on the tissue culture flask induces bio-inert storage of hematopoietic stem cells in umbilical cord blood.

Experimental

Carbonyldiimidazole (CDI)-activated Pluronic F127 was prepared from the procedure shown in Figure 1. Briefly, the purified Pluronic F127 (MW=13,388 g mol^{-1}, PEO_{99}-PPO_{65}-PPO_{99}) or F68 (MW=8,780 g mol^{-1}, PEO_{80}-PPO_{30}-PPO_{80}) was dissolved in dry THF and added dropwise to an excess amount of CDI in THF at room temperature under nitrogen atmosphere.[3,4] The solution was concentrated to a small volume under vacuum and poured into ethyl ether. The precipitate was collected by filtration. The CDI-activated Pluronic was obtained as white powder after recrystalization and drying under vacuum (yield 89%). CDI-activated Pluronic in methanol was inserted into the polylysine-coated flask. The flask was incubated for 24 h at 25 °C under a shaking incubator. The Pluronic-immobilized tissue culture flask (Pluronic-immobilized flask) was rinsed with methanol and with ultrapure water, subsequently. PL127-10 indicates the Pluronic-immobilized flask prepared on the concentration of CDI-activated Pluronic F127= 10 mg/mL.

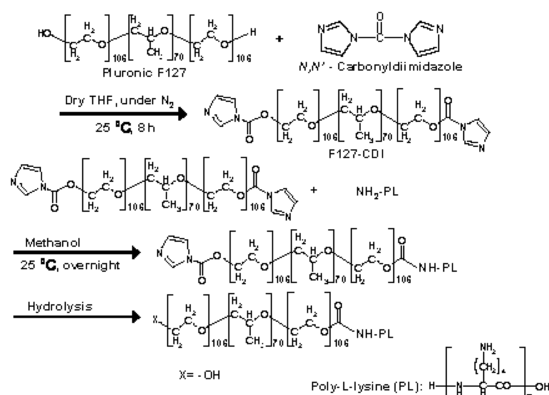

Figure 1. Preparation of Pluronic-immobilized flasks.

Results and discussion

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 cells on the Pluronic-immobilized flask increased up to 4-5 days, showing approximately constant 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.

Human umbilical cord blood was stored at 4 °C in the Pluronic-immobilized flask as well as a conventional polystyrene tissue cultureflask (PSt) and commercially available bio-inert flasks (HydroCell^R, RepCell^R and Nunc^R), and flow cytometric analysis of surface markers was performed on hematopoietic stem cells after cultivation. The surface of the RepCell^R flask is reported to be modified with poly(*N*-isopropylacrylamide) (PIPAAm) by electron-beam polymerization, while the surface of HydroCell^R flask is composed of a bio-inert material. Nunc^R flask was coated with MPC copolymer. Figure 2 shows the time dependence of the cell numbers of expressed CD34$^+$ hematopoietic stem cells in umbilical cord blood cultivated on several flasks. The number of cells expressing CD34$^+$ cells in umbilical cord blood cultivated on the polystyrene tissue culture flask, HydroCell^R flask and Nunc^R flask decreased significantly after one day of cultivation. The RepCell^R flask showed slightly better results for expression of hematopoietic stem cell markers. In contrast, the number of cells expressing CD34$^+$ in umbilical cord blood on the Pluronic-immobilized flask (PL127-10, PL68-10) was extremely higher than those obtained using other flasks studied in this study. It is concluded that the flexible and hydrophilic segments of Pluronic conjugated on the flask surface are the reason for the effective preservation of hematopoietic stem cells in the Pluronic-immobilized flask.

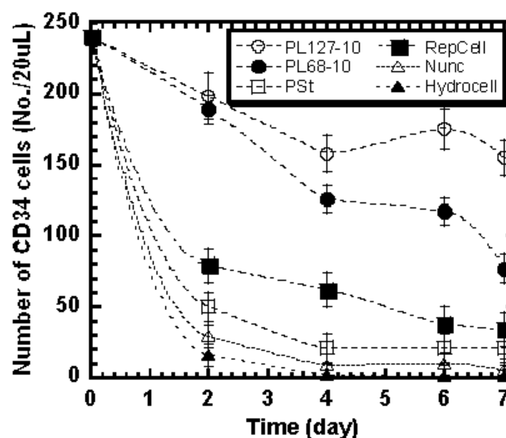

Figure 2. Time dependence of expression ratio of CD34+ of umbilical cord blood cells. The cells were incubated on a polystyrene tissue culture flask, a RepCell (PNIPAAm) flask, a HydroCell flask, Nunc (MPC) flask and Pluronic flask (PL127-10, PL68-10) at 4 °C.

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