Preservation of Hematopoietic Stem Cells from Umbilical Cord Blood Stored in a Surface Derivatized with Polymer Nanosegments

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Once cells are attached to the surface of a material, intracellular signals regulating their proliferation and differentiation are generated via interaction between specific receptors and cell signaling molecules adsorbed or expressed on the materials. However, the surfaces of materials on which cells do not proliferate, differentiate or de-differentiate have not yet been studied extensively.$^{1,2}$ These materials should be useful with or without a specific ligand interacting with specific cells (e.g., E-cadherin, N-cadherin, extracellular matrix and cell binding molecules) for the culture and or preservation of embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, mesenchymal stem cells and hematopoietic stem cells. Therefore, we developed a tissue culture flask with immobilized amphiphilic nano-segments of Pluronic F68 and F127 (Pluronic-immobilized flask) in this study (Fig. 1). The triblock copolymer Pluronic was composed of polyethylene oxide (PEO)-polypropylene oxide (PPO)-PEO triblocks, exhibits amphiphilic properties. When fibroblasts (L929 cells) were cultured in a Pluronic F127-immobilized flask, their morphology was mainly spherical, and they showed less spreading behavior than in Pluronic F68-immobilized flasks or conventional tissue culture flasks. This indicated that the Pluronic F127-immobilized flask provided a specific

![Reaction scheme](attachment:image.png)

**Fig. 1** Reaction scheme for the synthesis of CDI-activated Pluronic F68 and F127 and immobilization of CDI-activated Pluronic F68 and F127 on polylysine-coated flasks.
environment (i.e., bioinert) for the culture of these cells. Therefore, to evaluate the specificity of this flask, umbilical cord blood was preserved in a Pluronic-immobilized flask having several surface concentration of Pluronic and in conventional tissue culture flasks at 4°C (Fig. 2). We examined the effect of surface concentration of Pluronic on the number and survival of hematopoietic stem cells from umbilical cord blood stored in such flasks. The expression ratio of surface markers (CD34) on hematopoietic stem cells stored in Pluronic-immobilized flasks was significantly higher than that in polystyrene tissue culture flasks or commercially available bio-inert flasks (i.e., low cell-binding cultureware). This was due to the presence of flexible brush-like segments of Pluronic on the Pluronic-immobilized flask. A good correlation was found between the number of CD34+ cells and the ratio of viable CD34+ cells from cord blood in several flasks after five days of storage. Therefore, the high number of CD34+ cells was thought to have originated from the high viability of these cells stored in Pluronic-immobilized flasks. It was found that there was an optimal surface concentration of Pluronic on the Pluronic-immobilized flask surfaces for the preservation (high number and survival) of these stem and progenitor cells. The foregoing results were attributable to the high density of Pluronic nano-segments on the flask surface, limiting the movement of these flexible segments.

Fig. 2 No. of hematopoietic stem cells preserved in several dishes (right) and Schematic image of surface having nano-brush for the preservation of hematopoietic stem cells (left).

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References
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