MSC Separation on Bioactive Molecule-Immobilized Column

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Introduction

Mesenchymal stem cell (MSC) is the ideal cell source in regenerative medicine due to its multipotent and proliferative activity which are essential for treating the defective tissues. Separation and purification of MSCs from living body are critical step for autologous cell plantation and tissue engineering. FACS or Ficoll methods were generally selected for separating the MSCs, but the isolated cells are not homogeneous. Moreover, these methods have the disadvantage to require some reagents which allow the dissociation of the cells and the antibodies, and the cell damage during the purification procedure is another problem. The therapeutic effects of target tissue using MSCs were reported in clinical research, but these contaminations may result in unknown side effects.

To secure the safety of MSC plantation as a clinical treatment, it is necessary to develop novel procedure for separating the homogeneous cells. In this research, we develop a cell separation column that was immobilized with antibody against MSC. The cells injected to the column roll on the inner surface of the column under a flow condition like a rolling adhesion phenomenon of the leukocyte in the blood vessel [1]. Polyacrylic acid was grafted on the surface of the polyethylene or silicone tube by ozone-induced graft polymerization. The anti-human CD34 antibody was immobilized on the surface. The cell suspension of KG-1a (CD34 positive) or HL-60 (CD34 negative) passed through the column, and the number and surface marker pattern of the cells in each elution fraction were analyzed by FACS system. The MSCs derived from mouse bone marrow was also injected in the column and the number and pattern was evaluated by FACS.

Material and Methods

Cell culture

KG-1a (CD34 positive) cells were grown in culture dish in IMEM (Gibco-Invitrogen, Carlsbad, USA) supplemented with 10% fetal bovine serum (FBS) (Sigma, St. Luis, USA), penicillin (100U/ml), streptomycin (100ug/ml). HL-60 cells were grown in culture dish in RPMI (Gibco-Invitrogen, Carlsbad, USA) containing 20% FBS. The dishes were cultured in a humidified atmosphere containing 5%CO_2 at 37°C.

Isolation and culture of mesenchymal stem cells

Mesenchymal stem cells (MSCs) were corrected according to a protocol modified from Tropel et al. [2]. Mouse bone marrow (BM) was isolated from 10-12-weeks-old C57Bl/6 mice (Japan SLC, Inc., Japan) by flushing the marrow cavities. The MSCs were prepared by the BM cultured on fibronectin-coated Petri dish (BD Pharmingen, Pont-de-Claix, France) with alpha-MEM (Gibco-Invitrogen, Carlsbad, USA) containing 15% FBS, EGF 10ng/ml and PDGF-AA 10ng/ml (R&D Systems, Minneapolis, USA).

Preparation of anti-CD34 antibody immobilized column

Polyethylene (inner diameter:1mm) or silicone (inner diameter:0.5mm) tube was treated with ozone gas for 4 hours, dipped in 10% acrylic acid/methanol solution, and incubated at 60ºC. After 4 hours, the tube was washed with water. The graft polymerization was confirmed by the toluidine blue staining. To immobilize anti-CD34 antibody on the surface of tube, the tube which was preactivated with carbodiimide was filled with the antibody solution, and incubated at 37ºC for 15 hours. After the incubation, the tube was washed with PBS buffer. To prepare the control column, the carbodiimide-activated column was reacted with the 2-aminoethanol instead of the anti-CD34 antibody.

Cell separation

The cell suspension ($2x10^4$ cells, 10μL) was injected into the antibody-immobilized column, and PBS buffer was continuously flown into the tube. Each eluted fraction was collected and analyzed. The flow rate was 50μL/min between the fraction 1 to 5, and the rate was 600μL/min between fractions 6-20. The number and surface marker property of cells in collected fractions were analyzed by FACS system.

Results and Discussion

The density of immobilized antibody on the column was evaluated using horseradish peroxidase-labeled antibody. The antibody density of anti-CD34 immobilized column was about 200μg/m^2. To evaluate the cell-separation activity of the antibody-immobilized column, KG-1a or HL-60 cells were applied to the column, and the elution fractions were analyzed by FACS system. When the KG-1a cells were injected into the anti-CD34 immobilized column, about 50% of the injected cells were eluted as delayed fraction. The fractions were found to consist of the cells with high density of CD34 surface marker. In contrast, the delayed fraction was not observed in the case of HL-60 cells. Moreover, the fraction was not detected when the KG-1a or HL-60 cells injected into the control column. These results suggested that the cells in delayed fraction rolled on the column surface in the cell surface marker-specific manner under the flow condition. Next, the MSCs injected into the antibody immobilized column, and the elution profiles of stem cells were investigated. Even in this

case, the delayed fractions were detected as well as the result for the established cell line. The expression of CD34 surface marker of MSCs in the delayed fractions was evaluated by FACS analysis. The CD34 expression level of the MSCs included in the latter of delayed fractions was much higher than that in the first. This result indicated that the established column system can isolate the subpopulation of MSCs from the others, based on the surface marker density. In general, a population in the bone marrow cells which has the high adhesion properties were used as the MSCs. These isolated fractions of MSCs were then seeded on the fibronectin-coated Petri dish. The well adhesion property was confirmed on the cells in a specific fraction. As for this result, it was suggested that the surface marker property was closely related to the adhesion behavior. This permits us to consider that the differentiation features of the MSCs are also largely different depending on the each separated subpopulations. The detailed features about the differentiation are now investigation.

Conclusion

This study has shown that the subpopulations of MSCs were separated on the antibody immobilized column depending on the surface marker density. A subpopulation of the MSCs with very high adhesion property could be separated in a delayed fraction with high purity. The adhesion property of delayed fractions was largely different from the MSC's isolated from the conventional procedure. This antibody-immobilized column is useful to isolate the cells based on the density of surface markers.

Reference

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2. Philippe Tropel, Danièle Noël, Nadine Platet, Pierre Legrand, Alim-Louis Benabid and François Berger (2004), "Isolation and characterization of mesenchymal stem cells from adult mouse bone marrow" *Experimental Cell Research*, 295, pp.395-406.