Separation of mesenchymal stem cells on a novel ligand-immoblized material

Atsushi MAHARA, and Tetsuji YAMAOKA
Department of Biomedical Engineering, Advanced Medical Engineering Center,
National Cardiovascular Center Research

Statement of Purpose: Insufficient isolation and purification of mesenchymal stem cell (MSC) is the critical problem for autologous cell plantation and tissue engineering. In general, the MSCs are isolated from the bone marrow cells in FACS or MACS system. However, the isolated MSCs are not homogeneous, and these separation procedures have the disadvantage to require some impurities such as modified antibodies. Under the clinical use, it is important to avoid the addition of dispensable reagents as much as possible. To secure the safety of MSCs transplantation in the clinical trials, it is necessary to develop a novel system for isolating the homogeneous population of MSCs. In addition, the isolated cells should maintain the high and uniform property in terms their differentiation ability. To isolate the homogeneous cells, we were focused on the surface marker density that dramatically changes with their differentiation. We designed a cell separation column that was immobilized with antibody against the cell surface marker. When the cells injected to the column, the cells roll on the inner surface of the column under a flow condition like a rolling adhesion phenomenon of the leukocyte in the blood vessel. In this work, the separation profile of established cell line (KG-1a and HL-60 cell) or MSCs on this column, and the differentiation property of the isolated MSCs were investigated.

Methods: Mesenchymal stem cells (MSCs) were collected according to a protocol modified from that supported by Tropel et al.[2]. Murine bone marrow (BM) was isolated from 10-12-weeks-old C57Bl/6 mice (Japan SLC, Inc., Japan). The MSCs were prepared by the BM cultured on fibronectin-coated dish (BD Pharmingen, Pont-de-Clax, France) with alpha-MEM (Gibco-Invitrogen, Carlsbad, USA) containing 15% FBS, EGF 10ng/ml and PDGF-AA 10ng/ml (R&D systems, Minneapolis, USA). The cells were routinely cultured on fibronectin-coated dish in the medium. To prepare the cell separation column, poly(acrylic acid) was grafted on the surface of the polyethylene or silicone tube by ozone-induced graft polymerization. The anti-human CD34 antibody (DakoCytomation, Glostrup, Denmark) or anti-mouse CD34 antibody (Serotec Ltd., Oxford, UK) was immobilized on the surface with carbodiimide activation methods. The amount of immobilized antibody was measured using horseradish peroxidase-labeled antibody. The shear flow was given by syringe pomp (KD scientific Inc., Hollistone, MA) in the column. The overview of this system was shown in Figure 1. The cell suspension of KG-1a (CD34 positive) and HL-60 (CD34 negative) passed through the column and fractional number and surface marker pattern of cells in each fraction were analyzed by FACS system. The adherent cells fraction of murine bone marrow cells on fibronectin coated dish (crude MSCs) was also injected to the column, and the differentiation property of the MSCs in each fraction was evaluated.

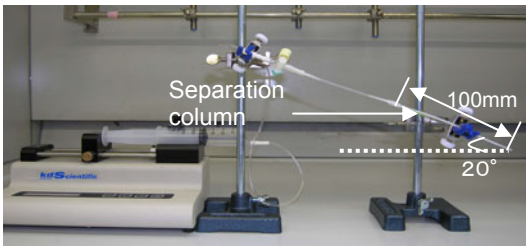

Figure 1. Photograph of cell separation system

Results/Discussion: The density of immobilized antibody was about 200μg/m^2. Two types of cell lines were applied to the column, and the elution profiles were evaluated. When KG-1a (CD34 positive) cells were injected into the anti-CD34 immobilized column, about 50% of the injected cells were eluted as delayed fraction. The delayed fractions contained cells with high density of CD34 surface marker, and the content was increased with the elution time. In contrast, the delayed fraction was not observed in the case of HL-60 (CD34 negative) cells. When the KG-1a or HL-60 cells injected into the unmodified column, all cells were found on the early fractions, and the delayed fraction was not observed. These results suggest that the cells in delayed fraction interacted with the column surface in the surface maker density-specific manner under the shear flow. In the next stage, the MSCs were injected into the column, and the elution profile was analyzed. Even in this case, the delayed fractions were observed as well as the results for the established cell line. The CD34 expression level of MSCs in the later fractions was much higher than that in the first. The isolated cells were incubated on the fibronectin-coated dish with osteoblastic differentiation medium for 4 days. The expression of osteoblastic marker gene was measured by real-time PCR analysis. The expression level of isolated cells was largely increased compared with that of the original MSCs. It could be concluded that the MSCs with different stage of cell differentiation were separated by the antibody-immobilized column.

Conclusions: The subpopulations of the cells could be separated on the antibody-immobilized column in surface marker density-specific manner. The MSCs at the different differentiation stages were separated on the column, suggesting that this separation mechanism is useful for the all transplantation and the fundamental research of MSCs.

References:

1. Mahara, A and Yamaoka, T., ASAIO J., 2006; 52:16A
2. Tropel, P., Noël, D., Platet, N., Legrand,P., Benabid, A. and Berger,F, 2004;295:395-406