Purification and Differentiation of Adipose-derived Stem Cells Separated from Adipose Tissue by A Membrane Filtration Method

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Statement of Purpose: Adipose-derived stem cells (ADSCs) are a promising cell source in regenerative medicine, of particular utility for cell therapies and tissue engineering, because adipose tissue can easily be harvested in large quantities compared to bone marrow. ADSCs are isolated from adipose tissue by liposuction and centrifugation followed by cultivation on cell culture dishes. The cultivation of cells derived from adipose tissue is necessary to purify ADSCs (i.e., "the culture method"). The culture process for the purification of ADSCs requires several days, at minimum. If ADSCs can be purified from adipose tissue in a short period of time (i.e., less than 2 hrs) by using a cell purification device such as the membrane filtration method, cell therapy and tissue engineering applications might become more efficient. We investigated the purification of ADSCs from a digested solution of adipose tissue by the membrane filtration method, and we compared the purity of ADSCs and the differentiation ability of ADSCs into osteoblasts and adipocytes after purification by the membrane filtration method and the conventional cell culture method.

Methods: Adipose tissue was isolated from the inguinal fat pads of four-week old ICR mice. The adipose-tissue cell solution was prepared from a conventional method.[1] The primary adipose-tissue cell solution was purified by the conventional culture method and the membrane filtration method. Two different filtration methods, i.e., batch-type filtration and perfusion-type filtration, were applied to purify ADSCs from the primary adipose-tissue cell solution. Two kinds of membranes were used in this study, (a) polyurethane foaming membranes (PU) with a pore size of 11 μm, and (b) Nylon mesh filters with a pore size of 11 μm [NY11]. The number of ADSCs in the permeate and recovery solutions was counted with flow cytometry measurements using CD73 and CD90 antibodies. ADSCs were seeded at 2 x 10^4 cells in DMEM culture medium containing 10% FBS, and incubated for 18 hrs. Then, the cells were cultured in adipogenic or osteogenic medium for up to 21 days.

Results: Adipose-derived stem cells (ADSCs) were purified from mice adipose-tissue cell solutions by the conventional culture method and the membrane filtration (i.e., batch-type filtration and perfusion-type filtration) method. The ADSCs expressing the mesenchymal stem cell marker CD73 were concentrated in a recovery solution through one sheet of polyurethane (PU) foaming membranes, and in a permeate solution through five sheets of Nylon mesh filters, by the perfusion-type filtration method (Fig. 1). This provided a concentration of cells expressing the marker that was 1.7 times higher than that of cells in the primary adipose-tissue cell solution. The ADSCs in the recovery solution that went through the PU foaming membranes but not through the Nylon mesh filters showed greater adipogenic and osteogenic differentiation ability than the cells contained in the primary adipose-tissue cell solution (Fig. 2). The perfusion-type filtration effectively recovered ADSCs with a greater ability to differentiate into adipocytes and osteoblasts than the cells recovered by batch-type filtration. ADSCs with adipogenic and osteogenic differentiation abilities tend to adhere to PU membranes but not to Nylon mesh filters when filtered by perfusion-type filtration. The relationship between the ratio of cells expressing the mesenchymal stem cell surface marker (i.e., CD73) and the adipogenic and osteogenic differentiation ability of the cells was also investigated.

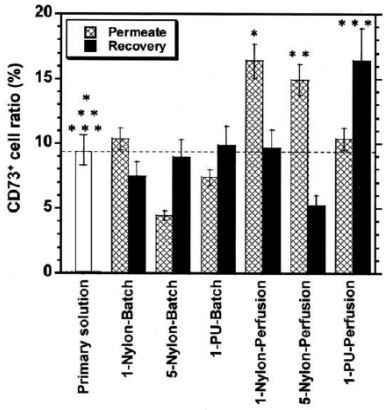

Figure 1. CD73 expression ratio of cells filtered through membranes.

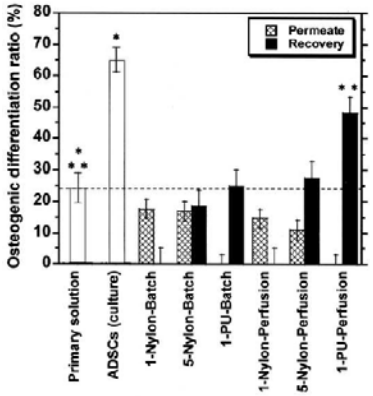

Figure 2. Osteogenic differentiation ratio of the adipose-tissue cell solution purified by the conventional culture method, and by membrane filtration methods.

Conclusions: ADSCs were successively purified from a mouse adipose-tissue cell solution by the membrane filtration method, which show high differentiation ability into adipocytes and osteoblasts. ADSCs can be obtained by the membrane filtration method in a short period of time, while the culture method requires several days to purify ADSCs.

1. Higuchi A. et al., J. Membr. Sci. in press.