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The isolation and differentiation of human adipose-derived stem cells using membrane filtration

Cheng-Han Wu [a,1], Fa-Kung Lee [b,1], S. Suresh Kumar [a], Qing-Dong Ling [c,d,**], Yung Chang [e], Yu Chang [f], Han-Chow Wang [g], Hui Chen [a], Da-Chung Chen [h], Shih-Tien Hsu [h], Akon Higuchi [a,c,i,*]

[a] Department of Chemical and Materials Engineering, National Central University, No. 300, Jhongda RD., Jhongli, Taoyuan 32001, Taiwan
[b] Department of Obstetrics and Gynecology, Cathay General Hospital, 280, Renai Rd., Sec. 4, Taipei, Taiwan
[c] Cathay Medical Research Institute, Cathay General Hospital, No. 32, Ln 160, Jian-Cheng Road, Hsi-Chi City, Taipei 221, Taiwan
[d] Institute of Systems Biology and Bioinformatics, National Central University, No. 300, Jhongda RD., Jhongli, Taoyuan 32001, Taiwan
[e] Department of Chemical Engineering, R&D Center for Membrane Technology, Chung Yuan Christian University, 200, Chung-Bei Rd., Chungli, Taoyuan 320, Taiwan
[f] Kaohsiung Medical University Hospital, No.100, Tzyou 1st Road, Kaohsiung 807, Taiwan
[g] Hungchi Women & Children's Hospital, No. 233, Yuanhua Rd., Jhongli, Taoyuan 320, Taiwan
[h] Taiwan Landseed Hospital, 77, Kuangtai Road, Pingjen City, Taoyuan 32405, Taiwan
[i] Department of Reproduction, National Research Institute for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan

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ABSTRACT

Human adipose-derived stem cells (hADSCs) were purified from a suspension of human adipose tissue cells (stromal vascular fraction) by the conventional culture method and by membrane filtration through polyurethane (PU) foam membranes. hADSCs can be obtained from a suspension of human adipose tissue cells using the membrane filtration method in less than 30 min, whereas the conventional culture method requires 5–12 days. hADSCs that express the mesenchymal stem cell markers CD44, CD73, and CD90 were concentrated in the recovery solution from the PU membranes; no hADSCs were isolated in the permeate. After filtration, the cells expressing the mesenchymal stem cell markers were 3–4.5 times more concentrated than in the initial suspension of human adipose tissue cells, with the level of concentration depending on the surface modification of the PU membrane. Cells expressing the stem cell-associated marker CD34 could be successfully isolated in the recovery solutions, whereas $CD34^+$ cells could not be purified by the conventional culture method. The hADSCs in the recovery solution demonstrated a superior capacity for osteogenic differentiation than did the cells in the suspension of human adipose tissue cells. These results suggested that the hADSCs with the capability for osteogenic differentiation adhered to the PU membranes.

1. Introduction

Stem cells are an attractive source of cells for tissue engineering and cell therapy (i.e., regenerative medicine using stem cells) because of their unique biological properties [1,2]. There are major ethical concerns regarding the use of embryonic stem cells (ESCs) in conventional research and as part of potential human therapies [3,4]. However, stem cells have been isolated from a variety of somatic tissues. These stem cells include hematopoietic stem cells (HSCs), isolated from umbilical cord blood, and mesenchymal stem cells (MSCs), which are isolated from bone marrow, umbilical cord blood, amniotic fluid and tissues such as fat tissue [5]. Adipose-derived stem cells (ADSCs) hold promise for regenerative medicine, particularly for cell therapies and tissue engineering applications [6–8].

Human ADSCs (hADSCs) express specific surface markers such as STRO-1 (a stromal cell surface antigen), CD29 (integrin β1), CD44 (receptor for hyaluronic acid and matrix proteins), CD73, CD90, CD105 (endoglin, receptor for transforming growth factor-β (TGF-β) and integrins), and CD166 (cell adhesion molecule) but are negative for CD14 (monocyte surface antigen) and CD45 (leukocyte surface antigen) [5]. These and other cell surface markers, although not

* Corresponding author. Department of Chemical and Materials Engineering, National Central University, No. 300, Jhongda RD., Jhongli, Taoyuan 32001, Taiwan. Tel.: +886 3 4227151x34257; fax: +866 3 280 4271.
** Corresponding author. Cathay Medical Research Institute, Cathay General Hospital, No. 32, Ln 160, Jian-Cheng Road, Hsi-Chi City, Taipei 221, Taiwan. Tel.: +886 3 4227151x34257; fax: +866 3 280 4271.
E-mail addresses: qdling@cgh.org.tw (Q.-D. Ling), higuchi@ncu.edu.tw, higuchi@gmail.com (A. Higuchi).
[1] These authors contributed equally to this work.

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unique to hADSCs, are used to isolate and/or characterize hADSCs using flow cytometry. hADSCs can be isolated from liposuction-derived adipose tissue by centrifugation followed by cultivation in cell culture dishes for at least one passage [9,10]. The cultivation of cells derived from adipose tissue is necessary to purify hADSCs (i.e., "the culture method" for the purification of hADSCs) because the adipose tissue also contains adipose cells and other cell types. Purifying hADSCs via the culture process requires 5–12 days. If hADSCs could be purified from adipose tissue in a shorter time period (i.e., less than 30 min) using a cell purification device such as that used in the membrane filtration method, cell therapy and tissue engineering applications using autologous hADSCs might become more efficient.

The membrane filtration method is a good candidate for the purification of stem cells because it is a rapid, simple method, and sterility can easily be maintained during the filtration process [11–15]. Furthermore, the membrane filtration method does not necessitate the use of antibodies, which are required for purifications that utilize magnetic-activated cell sorting (MACS) [16,17] or fluorescence-activated cell sorting (FACS) [18–20]. Antibodies are generally produced using animal-derived proteins and cells, which might lead to contamination with viruses or prions. Therefore, hADSCs should not be prepared using animal-derived materials, such as antibodies, to maintain the safety of these cells in clinical applications.

We previously reported the purification of ADSCs from mouse adipose tissue cell solutions (mADSCs) by the conventional culture method and the membrane filtration method (i.e., batch-type filtration and perfusion-type filtration) [13]. mADSCs expressing the mesenchymal stem cell marker CD73 were concentrated in the recovery solution after perfusion-type filtration through one sheet of a polyurethane (PU) foam membrane with a pore size of 11 μm [13]. After this purification, cells expressing CD73 were 1.7 times more concentrated than in the suspension of human adipose tissue cells (primary adipose tissue cell solution). The mADSCs in the recovery solution possessed superior adipogenic and osteogenic differentiation abilities [13]. Although experiments have been performed with mouse cells, the isolation of hADSCs from human adipose tissue by the membrane filtration method and the differentiation ability of these cells have not yet been reported. It is known that the characteristics of human stem cells are completely different from those of stem cells derived from other animals, such as mice. For example, it is extremely difficult to clone humans, but cloning other animals is not as difficult. Human ESCs and induced pluripotent stem cells (iPSCs) are more difficult to prepare and establish than murine ESCs and iPSCs [21]. Therefore, we purified hADSCs from a digested solution of human adipose tissue by the conventional cell culture method and by the membrane filtration method using unmodified polyurethane (PU) membranes and surface-modified PU membranes, and we compared the purities and the osteoblast differentiation abilities of the hADSCs after each purification.

2. Materials and methods

2.1. Preparation of the adipose tissue cell solution

The animal experiments in this study were approved by the ethics committees of National Central University, Taiwan Landseed Hospital, and Cathay Medical Research Institute. Adipose tissue was isolated from the fat pads of human patients (49–81 years, four persons) with informed consent. The adipose tissue cell solution was prepared as follows. The fat pads were carefully dissected and washed with phosphate-buffered saline (PBS) to remove blood and impurities. The adipose tissue was minced into small pieces (approximately 2 mm^3) and digested with 2.5 mg/mL of type I collagenase (C0130, Sigma–Aldrich, St. Louis, MO) at 37 °C for 60 min. The enzymatic activity was neutralized with Dulbecco's Modified Eagle's Medium (D5523, DMEM, Sigma–Aldrich, St. Louis, MO) containing 10% fetal bovine serum (04-001-1, FBS, Lo 551035, Biological Industries Ltd., Israel). The digested solution was centrifuged at 1200 × g for 7 min. The resulting cells were suspended for 2 min in erythrocyte lysis buffer (154 mM NH_4Cl, 20 mM Tris, pH 7.4) to remove the red blood cells, and the solution was subsequently neutralized with DMEM culture medium containing 10% FBS. The cell solution was centrifuged at 1200 × g for 7 min. The resulting cells were resuspended in DMEM culture medium containing 10% FBS to yield the primary adipose tissue cell solution (adipose tissue-derived stromal vascular fraction (SVF)).

The number of hADSCs in the primary adipose tissue cell solution was counted using flow cytometry with antibodies to CD34 (IM1870U, FITC Mouse Anti-Human CD34, Beckman Coulter, Inc, Marseille, France), CD44 (IM1219U, FITC Mouse Anti-Human CD44, Beckman Coulter, Inc, Marseille, France), CD73 (550257, PE Mouse Anti-Human CD73, BD Biosciences, San Jose, CA), CD90 (IM1840U, PE Mouse Anti-Human CD90, Beckman Coulter, Inc, Marseille, France), and their isotype controls (733179, PE Mouse Anti-Human IgG1 and 41116015, FITC Mouse Anti-Human IgG1, Beckman Coulter, Inc, Marseille, France). The total cell number in the primary adipose tissue cell solution was also counted by flow cytometry (Coulter EPICS™ XL, Beckman Coulter, Inc, Marseille, France) after staining with 7-AAD (A07704, Beckman Coulter, Inc, Marseille, France). The primary adipose tissue cell solution was purified by the conventional culture method [22–25] and the membrane filtration method.

2.2. Purification of hADSCs by the culture method

In the culture method, the cells in the primary adipose tissue cell solution (SVF) were plated in tissue culture flasks (Falcon®, BD Biosciences, USA) at approximately 30,000 cells/cm^2 in DMEM containing 10% FBS at 37 °C in a 5% CO_2 incubator. Upon reaching approximately 80% confluence (5–12 days), the cells were trypsinized with a 0.25% trypsin-EDTA solution (25200-056, Invitrogen Corporation, Carlsbad, CA), followed by centrifugation at 1500 rpm for 5 min. The hADSCs thus obtained were suspended in DMEM containing 10% FBS and were used for analysis and differentiation experiments (first passage hADSCs). The number of hADSCs in the cell culture medium at the first passage was counted by flow cytometry using antibodies against CD34, CD44, CD73, and CD90 and their isotype controls. The total cell number in the cell culture medium at the first passage was also counted by flow cytometry.

2.3. Surface modification of PU foam membranes by plasma polymerization

The base membranes used for the chemical modification were polyurethane (PU) foam membranes with a pore diameter of 11 μm (Ruby Cell S, Toyo Polymer Co., Ltd.). PU foam membranes containing an epoxy group (PU-epoxy) were prepared by

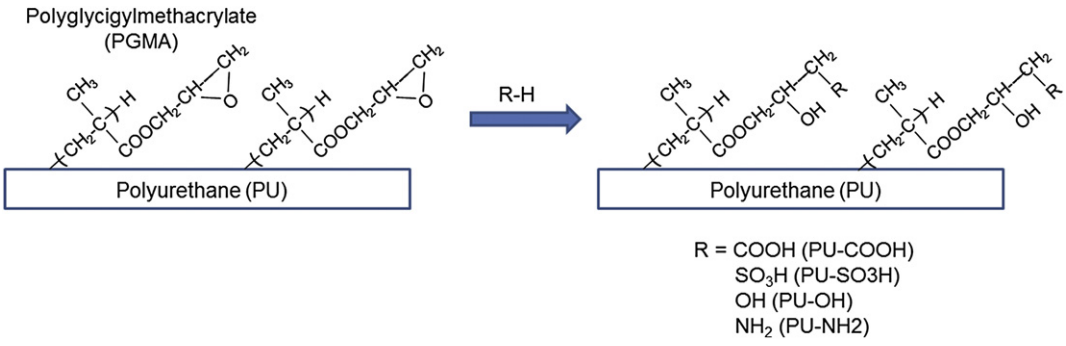

Fig. 1. Reaction scheme for synthesizing the surface-modified PU membranes.