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Proliferative and phenotypical characteristics of human adipose tissue–derived stem cells: comparison of Ficoll gradient centrifugation and red blood cell lysis buffer treatment purification methods

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Abstract

Background aims. Adult human subcutaneous adipose tissue harbors a multipotent stem cell population, the so-called human adipose tissue–derived mesenchymal stromal cells (AT-MSCs). These cells are able to differentiate *in vitro* into various cell types and possess immunomodulatory features. Yet procedures to obtain AT-MSCs can vary significantly. The two most extensively used AT-MSC purification techniques are (i) density gradient centrifugation using Ficoll and (ii) red blood cell (RBC) lysis buffer treatment of the stromal vascular fraction. In the context of potential clinical cell therapy, the stem cell yield after purification and upon consecutive passages, as well as the purity of the obtained cell population, are of utmost importance. *Methods.* We investigated the expansion capacity and purity of AT-MSCs purified by both procedures immediately after isolation and upon consecutive passages. We also investigated possible purification-dependent differences in their expression of immune-inhibitory factors and cell adhesion molecules. *Results.* We found that RBC lysis buffer treatment is a more robust and easier method to purify AT-MSCs than density gradient fractionation. However, the resulting AT-MSC-RBC population contains a significantly higher number of $CD34^+$ cells, particularly during the first passages after plating. From passage 4 onward, no significant differences could be observed between both populations with respect to the immunophenotype, expansion capacity and expression of immune inhibitory factors and cell adhesion molecules. *Conclusions.* Our data show that RBC lysis buffer treatment may be a good alternative to density fractionation, providing a faster, more robust and easier method to purify AT-MSCs with biologically preserved characteristics.

Key Words: *adipose tissue, adult stem cell, density gradient centrifugation, Ficoll, mesenchymal stromal cell, red blood cell lysis, stromal vascular fraction*

Introduction

Postnatal stem cells have been identified in most human tissues/organs (1,2). Mesenchymal stromal cells (MSCs) represent, together with hematopoietic stem cells, the most widely used class of postnatal stem cells. According to the International Society for Cellular Therapy, MSCs can be identified by their plastic adherent properties, immunophenotype ($CD73^+$, $CD90^+$, $CD105^+$, $CD11b/14^-$, $CD19/CD73b^-$, $CD34^-$, $CD45^-$ and $HLA\text{-}DR^-$) and differentiation potential into adipocytes, chondrocytes and osteoblasts (3). MSCs have been isolated from various human adult sources including, but not limited to, bone marrow (4), liver (5), adipose tissue (6) and umbilical cord (7,8). Moreover, MSCs are able to differentiate *in vitro* into various cell types and possess immunomodulatory features (9,10). These properties make MSCs an attractive tool for regenerative medicine in the context of cell therapy.

The best characterized MSCs are derived from bone marrow (BM). However, the technique to isolate BM is invasive and yields a low quantity of MSCs. Adipose tissue (AT), on the other hand, has emerged over the years as an alternative source for human MSCs (11). AT, like BM, is derived from the embryonic mesoderm and contains a stromal

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vascular fraction (SVF) that harbors a stem cell population termed adipose tissue–derived mesenchymal stromal cells (AT-MSCs) (6,12). These AT-MSCs can be easily isolated from routine liposuction (lipoaspirate) and reconstructive surgery (lipectomy) waste materials using a collagenase-based digestion procedure (13). Under proper conditions, cells within the SVF subsequently adhere to plastic tissue culture dishes and exhibit a fibroblast-like appearance (6,14). It is generally agreed that the SVF is a heterogeneous cell population that harbors not only AT-MSCs but also fibroblasts, endothelial cells, pericytes, smooth muscle cells and circulating cell types, such as immune cells and hematopoietic stem cells (15,16). For example, early passages of AT-MSCs often express the hematopoietic stem cell marker $CD34^{+}$ (17,18). As a consequence, several distinct purification techniques are currently being used to enrich the AT-MSC subpopulation from the SVF. The most extensively used purification techniques are based on either density gradient centrifugation or red blood cell (RBC) lysis buffer treatment of the SVF (19). Other techniques include mechanical dissociation (20) and more advanced and expensive methods such as the use of immunomagnetic beads coated with specific antibodies, including CD34, CD105 and CD271 (21,22), fluorescence-activated cell sorting (FACS) (23) and approaches based on high aldehyde dehydrogenase activity (17,24). However, in the context of clinical cell therapy, the stem cell yield after purification and upon consecutive passages, as well as the purity of the obtained cell population, are of key importance. Therefore, we investigated the proliferative capacity and the purity of the resulting AT-MSCs purified by the two most frequently used methods.

Besides their multi-lineage differentiation potential, AT-MSCs also exhibit immunomodulatory properties. More specifically, AT-MSCs are able to inhibit the activation and proliferation of immune cells (25) due to cell-cell contacts and release of soluble immunosuppressive factors, such as hepatocyte growth factor (HGF), leukemia inhibitory factor (LIF) and prostaglandin (PG) E2 (9,10,25). As such, AT could represent an alternative, easily accessible source of MSCs to BM for clinical cell therapy, in particular for the treatment of graft-versus-host disease and autoimmune disorders (26).

Within this context, we also investigated whether the purification procedure affects the gene expression of the major regulatory factors reported to be involved in AT-MSC inhibitory effects including the cell adhesion molecules (CAMs) intercellular adhesion molecule (ICAM) 1 and vascular cell adhesion molecule (VCAM) 1. The immune inhibitory factors HGF, LIF, heme oxygenase (HMOX) 1, prostaglandin-endoperoxide synthase (PTGS) 1 and PTGS2 were also studied.

To the best of our knowledge, no work has thus far been reported that evaluates whether the proliferation capacity of AT-MSCs and their expression of CAMs and immune inhibitory factors are affected by the purification method used.

Methods

AT collection

AT (lipoaspirate) was collected after obtaining informed consent from three male and nine female patients (age range 25–45 [37 ± 8] years) undergoing elective liposuction in cooperation with the Department of Plastic Surgery of the UZ-Brussels (Brussels, Belgium) and the ATLAS clinic (Brussels, Belgium).

Isolation and culture of AT-MSCs

Human AT-MSCs were isolated from 12 patients according to a modified protocol from Oedayrajsingh-Varma *et al.* (13). Importantly, both purification methods were applied to all patients in parallel. In brief, 125 mL of liposuction material was extensively washed with equal volumes of phosphate-buffered saline (PBS) to remove erythrocytes. Centrifugation was carried out for 3 min at 600*g*. The adipose tissue samples were incubated for 45 min at 37°C with dissociation medium (1:1), that is, 125-mL aliquots of fat + 125 mL of dissociation medium. The latter consisted of 1% (v/v) bovine serum albumin (BSA) (Sigma-Aldrich, Diegem, Belgium) and 1 mg/mL collagenase A (Roche Applied Science, Vilvorde, Belgium) in PBS. The digested tissue was then passed through a mesh filter to remove connective tissue debris. Subsequently, the filtrate was centrifuged for 10 min at 600*g* (4°C), and the supernatant was removed. In the classical Ficoll gradient protocol, the cells were suspended in 50 mL PBS supplemented with 1% (v/v) BSA and centrifuged again for 10 min at 600*g* (4°C), after which the pellet was resuspended in 30 mL of PBS, supplemented with 1% (v/v) BSA. The cell suspension was then carefully brought on top of 15 mL of Ficoll gradient solution (Sigma-Aldrich) and centrifuged for 20 min at 1000*g* (4°C). Upon centrifugation, the top layer was removed and the AT-MSC-Ficolls (Fs) collected in 50 mL PBS supplemented with 1% (v/v) BSA. The cell suspension was centrifuged for 10 min at 600*g* (8°C), after which the supernatant was removed and total AT-MSC-Fs were counted using a hematocytometer. Cell viability was assessed using a 0.4% (w/v) trypan blue dye solution (Sigma-Aldrich).