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Cryopreservation of whole adipose tissue for future use in regenerative medicine

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ABSTRACT

Background: Human adipose tissue (AT) is an ideal stem cell source for autologous cell-based therapies. The preferred setting for tissue engineering and regenerative medicine applications is the availability of clinically acceptable off-the-shelf cells and cell products. As AT is not always available for use, cryopreserved tissue represents an alternative approach. The aim of the present study was to compare the different properties of mesenchymal stem cells (MSCs) isolated from cryopreserved AT. We have measured cell recovery, viability, phenotype, proliferative potential, and differentiation into mesenchymal (adipogenic, osteogenic, chondrogenic) and nonmesenchymal (neuron-like cells) lineages.

Materials and methods: AT ($n = 10$) was harvested from donors and either processed fresh or cryopreserved in liquid nitrogen dewars. Both fresh and thawed tissues were enzymatically digested. MSCs were analyzed by fluorescence-activated cell sorting for CD3, CD14, CD19, CD34, CD44, CD45, CD73, CD90, and CD105 expression. Growth characteristics of both groups were investigated for population doublings, doubling time, saturation density, and plating efficiency. MSCs derived from fresh and thawed tissues were assessed for differentiation potential both qualitatively and quantitatively.

Results: Adherent cells from fresh and thawed tissues displayed similar fibroblastic morphology. Cryopreservation did not alter expression of phenotypic markers. Similarly, the proliferative potential of MSCs was not compromised by cryopreservation. Furthermore, cryopreservation did not alter the differentiation capability of MSCs as determined with histochemistry, immunofluorescence, and real time reverse transcriptase-polymerase chain reaction.

Conclusions: We conclude that human AT could be successfully cryopreserved for future clinical application and the recovered MSCs were equivalent in functionality to the freshly processed MSCs.

1. Introduction

Human adipose tissue (AT) as a mesenchymal stem cell (MSC) source is more practical than bone marrow due to limitations including but not limited to bone marrow's painful acquisition, low MSC yield, need for anesthesia, and the often-older age of the donor. AT is widely distributed throughout the body and is routinely discarded during liposuction and other

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surgical procedures (such as cosmetic surgery). Stem cell banks offer the opportunity to preserve stem cells in viable conditions for prolonged periods until needed by the donor [1,2].

MSCs are defined by plastic adherent growth, positive expression of certain membrane molecules (CD44, CD73, CD90, CD105) together with a lack of expression of hematopoietic lineage markers (CD3, CD14, CD19, CD34, CD45), and differentiation into tissues developing from mesoderm (such as fat, bone, and cartilage). A high proliferation rate and multilineage differentiation potential makes MSCs an ideal cell source for tissue repair [3], hematopoiesis support [4], and immunomodulation [5].

Tissue engineering and regenerative medicine offers attractive therapeutic options for the treatment of various diseases and disorders. Many of these diseases could be treated by using stem cells from a patient's own body. AT, which is a rich source of MSCs, offers such possibility for autologous transplantation. However, MSCs cannot always be obtained in time to address situations where patients might benefit from such therapies. It is, therefore, important to preserve cells to be readily available at the point of care when a specific clinical application is recognized. Cryopreservation is the most reliable way for preserving cells and tissues to be used in future to augment or recover the function of damaged tissues and organs. In the present study, we sought to examine whether MSCs isolated from cryopreserved AT were functionally equivalent to freshly isolated MSCs.

In this study, MSCs derived from fresh and frozen AT were characterized by flow cytometry using a panel of standard cell surface molecules [6]. Growth characteristics of fresh and frozen MSCs were compared using the parameters of population doublings (PDs), population doubling time (DT), saturation density, plating efficiency, and attachment efficiency. Differentiation into cells of mesenchymal and nonmesenchymal lineages was also analyzed qualitatively and quantitatively using cytochemistry, real-time reverse transcriptase-polymerase chain reaction (RT-PCR), and immunofluorescence staining. The results of the present study indicated that cryopreserved AT-MSCs were functionally equivalent to MSC obtained from fresh AT.

2. Material and methods

2.1. Collection and cryopreservation of AT

All samples were obtained with written consent from the donors according to the requirements of the local Institution Review Board. AT samples ($n = 10$) were obtained from local surgeons performing a scheduled liposuction procedure. The lipoaspirates were either prepared for cryopreservation in 5 mL cryotubes or processed within 24 h of collection (referred to as fresh samples). For cryopreservation, 2 mL of washed tissue slurry was directly placed in a 5 mL cryotube and 2 mL of precooled dimethyl sulfoxide solution (70% basal medium, 20% serum, 20% dimethyl sulfoxide) was added drop wise in the cryotube. The cryotube was then mixed. Furthermore, the cryotube was spun using a tube rotator at 4°C for 30 min followed by cryopreservation in a controlled rate freezer to −180 C before final submersion in liquid nitrogen. Samples were stored frozen for at least 1 wk before thawing and analyses. The frozen tissues were thawed rapidly in a 37 C water bath. Immediately after thawing, the cryopreservation solution was diluted with expansion medium (α-MEM) supplemented with fetal bovine serum (FBS) in a 15 mL tube. The cryotubes were spun in a tube rotator for 30 min and digested to obtain cells (see below) similarly as processed fresh samples.

2.2. Isolation and expansion of MSCs

MSCs from fresh and thawed ATs were obtained by enzymatic digestion as follows [7]. The tissue slurry was digested with 0.2% type IV collagenase and incubated at 37 C for 15 min. MSC expansion medium consisted of α-MEM media supplemented with 10% FBS and 1% each of nonessential amino acids, sodium pyruvate, L-glutamine, and streptomycin/penicillin solution. Expansion medium was added to the digested tissue to neutralize collagenase. The infranatant was centrifuged at 150*g* for 10 min to obtain cells. Cells were plated in 25 cm^2 culture flasks and maintained in a humidified atmosphere at 37°C with 5% CO_2. After 2 d of culture, nonadherent cells were removed by changing the medium. Medium was changed twice weekly thereafter.

2.3. Cell surface antigen profile of MSCs

Cell surface marker expression [6] of cultured fresh and thawed cells was analyzed by fluorescence-activated cell sorting. Single cell suspensions of 1×10^5 cells were prepared from fresh and cryopreserved cultures. The cells were incubated with the following antibodies conjugated with fluorochromes (AF: alexa fluor, PE: phycoerythrin, APC: allophycocyanin, and FITC: fluorescein isothiocyanate): AF-CD3, PE-CD14, APC-CD19, PE-CD34, APC-CD44, FITC-CD45, PE-CD73, AF-CD90, and APC-CD105 for 30 min at 4°C. Samples were analyzed using aLSR II flow cytometer (BD Biosciences) using fluorescence-activated cell sorting DIVA software (BD Biosciences). Unstained cells were used to establish flow cytometer settings. Debris and autofluorescence were removed by using forward scatter. At least 10,000 gated events were used for each analysis.

2.4. Growth characteristics

To compare the growth characteristics of fresh and frozen MSCs, the plating efficiency, maximum PDs, DT, and saturation density were measured.

2.4.1. Plating efficiency

The capacity of fresh and frozen AT-derived MSCs (AT-MSC) to form colonies was evaluated as described earlier by Choudhery *et al.* [8]. Briefly, at passage 1, both types of cells were seeded in 25 cm^2 culture flasks at 20 cells per cm^2 and propagated in expansion medium supplemented with 10% FBS. Two weeks after culturing, resulting colonies were fixed with methanol and stained with crystal violet (0.1%). Colonies with >30 cells were examined directly by microscopic observation of flasks and counted manually. To score colonies, each MSC sample was plated in triplicate and the colonies were