

Isolation of adipose tissue-derived stem cells

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Abstract: Mesenchymal stem cells are being used increasingly in cell-based therapies. Adipose tissue is an important source of mesenchymal stem cells and is also routinely used in research on lipid metabolism and obesity. Their high expansion potential and the ease of isolation make these cells an attractive cell source for regenerative therapies. The objective of this review is to give detailed information about the isolation, expansion, and clinical use of these cells in veterinary medicine.

Key words: Mesenchymal stem cells, adipose tissue, isolation

1. Introduction

Adipose tissue is found in all vertebrates including humans. Adipose tissue is a special type of connective tissue in which adipocytes predominate. According to the anatomical region of the body, either dense or loose connective tissue is found in between adipose cells. This connective tissue is composed of fibroblasts, connective tissue fibers, blood and lymphatic vessels, and nerves (1). One gram of adipose tissue contains approximately 350,000 multipotent cells referred to as preadipocytes and approximately 5000 adipose tissue-derived stem cells. This number is 35-fold greater than the number of stem cells available in bone marrow. In other words, adipose tissue contains a much higher number of stem cells than bone marrow (2,3). Furthermore, recent research suggests that obesity, also known as the excessive accumulation of fat in the body, could be a stem cell disease (4,5). Adenovirus-36 has been shown to have an effect on stem cells and convert them into adipocytes (4,5). In veterinary medicine, stem cell treatment is performed with the use of not only adipose tissue-derived stem cells, but also bone marrow-derived stem cells. These cells are used mostly for the treatment of tendon and ligament injuries in horses and muscle and skeletal diseases in dogs, as well as for cartilage and joint damage (6,7). In a previously conducted study, the adipogenic differentiation and expansion potential of adipose tissue-derived stem cells was compared using tissue samples taken from young and old dogs. It was concluded that stem cells isolated from young dogs had a potential of rapid expansion (8). A recent review supported

the suggestion that adipose-derived stem cells from aging and ailing donors have a diminished proliferative and proangiogenic potential due to hypoxic conditions (9).

In clinical cell-based therapies, the new trend is to use the own stem cells of each organ (10). The fine structure and differentiation features of bone marrow-derived mesenchymal stem cells have been investigated by transmission electron microscopy and scanning electron microscopy, and it has been found that these cells are in continuous communication, which is very important for signaling (11,12).

Adipose tissue is an important source of multipotent mesenchymal stem cells. As we know, five different types of adipose tissue exist. These are bone marrow, brown, mammary, mechanical, and white adipose tissue. Each source has a distinct biological function. Bone marrow adipose tissue serves as an energy reservoir and cytokine source for osteogenic and hematopoietic events. Brown adipose tissue generates heat, has an abundant number of mitochondria, and is available around major organs (heart, kidney, aorta, gonads) in newborn infants but disappears as the body matures. Mammary adipose tissue provides nutrients and energy during lactation. Mechanical adipose depots, such as retroorbital and palmar fat pads, provide support to the eyes, hands, and other critical structures. White adipose tissue provides energy to the body and protects the organs from damage (13).

Due to ethical limitations concerning the source of embryonic stem cells, studies mainly focus on mesenchymal stem cells. The clinical use of adipose

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tissue-derived mesenchymal cells for treatment is rather common. Given their therapeutic use, the safety, efficacy, reproducibility, and quality of these cells are of importance. Therefore, isolation and cell expansion protocols have been developed for adipose tissue-derived mesenchymal cells. Adipose tissue-derived stem cells are considered as the most readily available type of stem cells, which are characterized by high reproducibility and a high potential of differentiation into bone, muscle, and cartilage cells, and they can be used in allogeneic stem cell treatment due to their low immunogenicity (7,14,15).

The high prevalence of obesity today has led to an increase in the number of studies carried out on brown and white adipose tissue and fat metabolism. Owing to its accessibility by surgery and its being a source of mesenchymal stem cells, adipose tissue is an important material for biomedical research. It has been reported that adipose tissue-derived mesenchymal cells differentiate into hepatocytes, osteoblasts, chondrocytes, myocytes, and nerve cells, and even into adipocytes under favorable conditions (14). Adipose tissue-derived stem cells are similar to bone marrow-derived stem cells in that they are mesenchymal stem cells, which adhere to plastic and have a potential of differentiating into osteocytes, chondrocytes, and adipocytes. For the characterization of these cells, it is required that they express cell surface markers CD105, CD73, and CD90. Recently the International Federation for Adipose Therapeutics and Science and the International Society for Cellular Therapy proposed criteria for defining adipose tissue-derived cells. Accordingly, the following surface markers should be investigated: +CD13, CD29, CD44, CD90, and CD105 (>80%) and -CD14, CD31, CD45, CD144, and CD235a (<2%) (9). CD34 is another marker that is of importance in adipose tissue mesenchymal stem cells. Recent knowledge about this marker includes its positivity in initial isolation procedures of adipose tissue stem cells and negativity after the first passage. At present it is not clear why this marker disappears after the first passage. However, it is proven that if a tissue is of adipose origin then this marker must be present in the analysis (16). Prominent characteristics of adipose tissue stem cells are immunomodulatory effects such as regulating the function of T cells, antiinflammatory cytokine expression, and time extension of the vitality of allotransplants. Thus, adipose tissue stem cells have been shown to suppress allogeneic lymphocytes in both in vitro and in vivo conditions (15,17–19). Adipose tissue stem cells do not retain MHC-II molecules, T helper cells and B cells, CD80, CD86, or CD40 on the surface. Related to these properties they are preferred in the treatment of immune disorders such as Crohn disease. In addition, due to their low immunogenicity, as well as in bone disease they can also be used in allogeneic stem cell therapy (15,20). In cell-

based therapy abundant numbers of cells are necessary. Therefore, it is necessary to replicate the adipose tissue stem cells prior to clinical application. Cells are passaged in appropriate media for proliferation. During passage of the cells to promote adhesion and proliferation fetal bovine serum (FBS) is used. However, in clinical trials in human medicine, animal-origin reagents are avoided and allogeneic human serum (allo-HS), autogenic human serum (auto-HS), and platelet-derived supplements were investigated. Removal of animal-origin reagents provides a high level of safety for the patient for cell transplant (15).

In adipose tissue stem cells, osteogenic, chondrogenic, and adipogenic differentiation capacity are optimized, and thus by providing differentiation protocols for each activity capacity can be increased. One reason for the decrease in the differentiation potential could be a decrease of the adhesion surface of the cell. In addition, there is a need for powerful nutrient-rich media for differentiation (15,21,22). In adipose tissue stem cells, three-way (trilinear) differentiation potential could be shown by oil red O, Alcian blue, and von Kossa staining (15).

As the mesenchymal stem cell-related information increases, the possibility of the use of these cells in regenerative medicine increases. This is particularly true for muscle and skeletal tissue; researchers have published several reports of the use of mesenchymal stem cells in orthopedic disorders.

Application of therapies based on autologous cell transplantation is recommended due to the multiple therapeutic potentials of these cells and low immunogenicity. However, a large part of the proposed therapy that requires prior replicating cell populations in vitro could adversely affect the phenotype of the cell as it moves slowly.

Single-surgery therapies, based on the isolation of autologous mesenchymal stem cells and their reintroduction into the site of injury within a short time period, not only reduce costs but also shorten the recovery period (23). Given their accessibility, their abundance in adipose tissue, and their osteogenic, adipogenic, and chondrogenic differentiation potential, human adipose tissue-derived stem cells isolated from the stromal vascular fraction of lipoaspirates are particularly suitable for use in single-surgery strategies (24).

In a previous study (23), cells derived from the stromal vascular fraction of adipose tissue were sorted on the basis of the expression of liver/bone/kidney alkaline phosphatase (ALP) levels with an aim to obtain cell subpopulations with enhanced osteogenic potential. For this purpose, a custom-designed molecular beacon for ALP was used in combination with fluorescence-activated cell sorting. High yields of cell subpopulations with a significantly enhanced osteogenic potential, as compared to unsorted

stromal vascular fraction cells and surface-marker sorted adipose stem cells, were obtained with this approach. Thus, cells offering an increased therapeutic potential for bone regeneration therapy were obtained.

2. Isolation and differentiation protocols for adipose stem cells

Adipose tissue is obtained from patients under general anesthesia and is transported to the laboratory in sterile tubes filled with Dulbecco's modified Eagle medium (DMEM) (Lonza, Belgium). The site of origin is also important for the final cell yield. The inguinal, gluteal, and extraabdominal fat tissue results are conflicting. Intraabdominal cell isolation and expansion results are more promising because they are more resistant to apoptosis (9). In the laboratory, the tissues are cut into small pieces (Figures 1A and 1B) in a sterile petri dish under laminar flow, placed into T25 flasks, and expanded by the explant culture method. Subsequently, the tissue culture flasks are placed in a 5% CO₂ incubator for 15 min with the addition of 77% DMEM (Lonza) growth medium containing 10% FBS (Lonza), followed by 2% L-glutamine (Lonza), 1% penicillin, streptomycin, and amphotericin

(Biological Industries, Israel). For dissociation of the tissue, collagenase treatment is another method of choice. Compared to the explant culture method mentioned above, the enzymatic treatment has higher cell yield but is a more invasive way of isolating cells (3,9).

The medium is replaced every 3 days and cell growth is observed under an inverted microscope (Olympus CX45). Once 70% confluence is reached (Figure 2), the cells are passaged at a ratio of 1:2, and prior to passaging the number and viability of the cells are checked. At the end of the third passage, the cells are subjected to adipogenic (fat), osteogenic (bone), and chondrogenic (cartilage) differentiation. For adipogenic differentiation, adipocyte differentiation basal medium and supplements (standard medium high-glucose DMEM (10% FBS), 0.5 mM 3-isobutyl-1-methylxanthine, 1 µM dexamethasone, 10 µg/mL insulin, 0.5 mM indomethacin (Sigma-Aldrich, Switzerland)) (25); for osteogenic differentiation, osteocyte differentiation basal medium (DMEM-LG, 0.05 mM ascorbate-2-phosphate, 100 nM dexamethasone, and 10 mM sodium β-glycerophosphate (Sigma-Aldrich)) (26); and for chondrogenic differentiation, chondrocyte differentiation basal medium (high-glucose DMEM containing 6.25 µg/mL insulin-transferrin-selenious acid, 0.1 mM ascorbate-2-phosphate, 10⁻⁷ M dexamethasone, 1.25 mg/mL bovine serum albumin, 5000 IU/mL penicillin, 5000 µg/mL streptomycin, 50 µg/mL ascorbate 2-phosphate, and 100 nM dexamethasone and 10 ng/mL human transforming growth factor) (26) are used (Figure 3).

At the end of the third week, oil droplets, calcium deposits in the extracellular matrix, and cartilaginous differentiation are observed (24,27).

As a result, the development of safe and effective *in vitro* methods that can be used for the isolation and expansion of adipose tissue-derived stem cells is of critical importance for the extension of cell-based therapies.

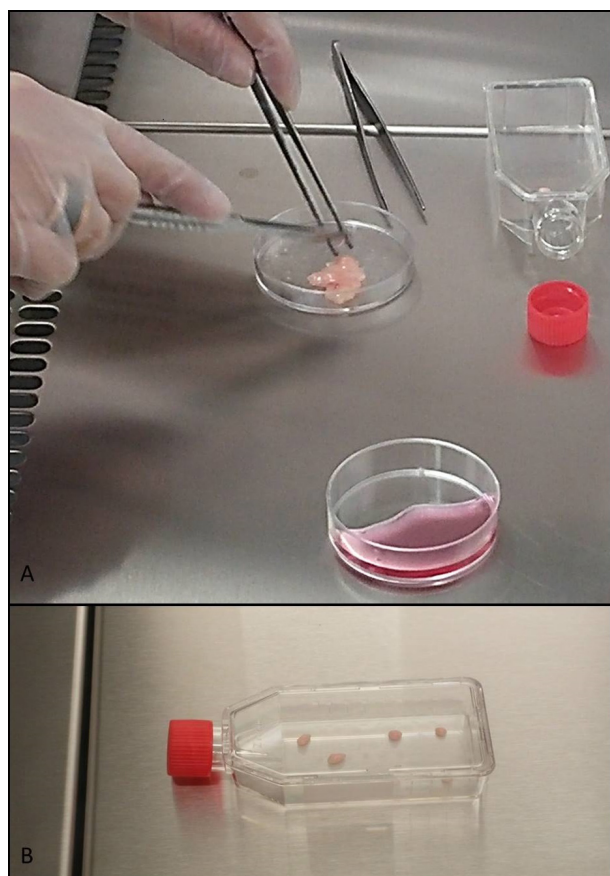


Figure 1. Extraction of adipose tissue from dog (A, B).

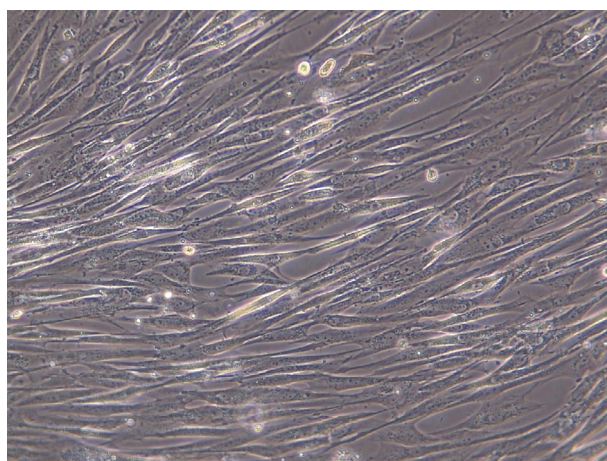


Figure 2. Appearance of a confluent flask.

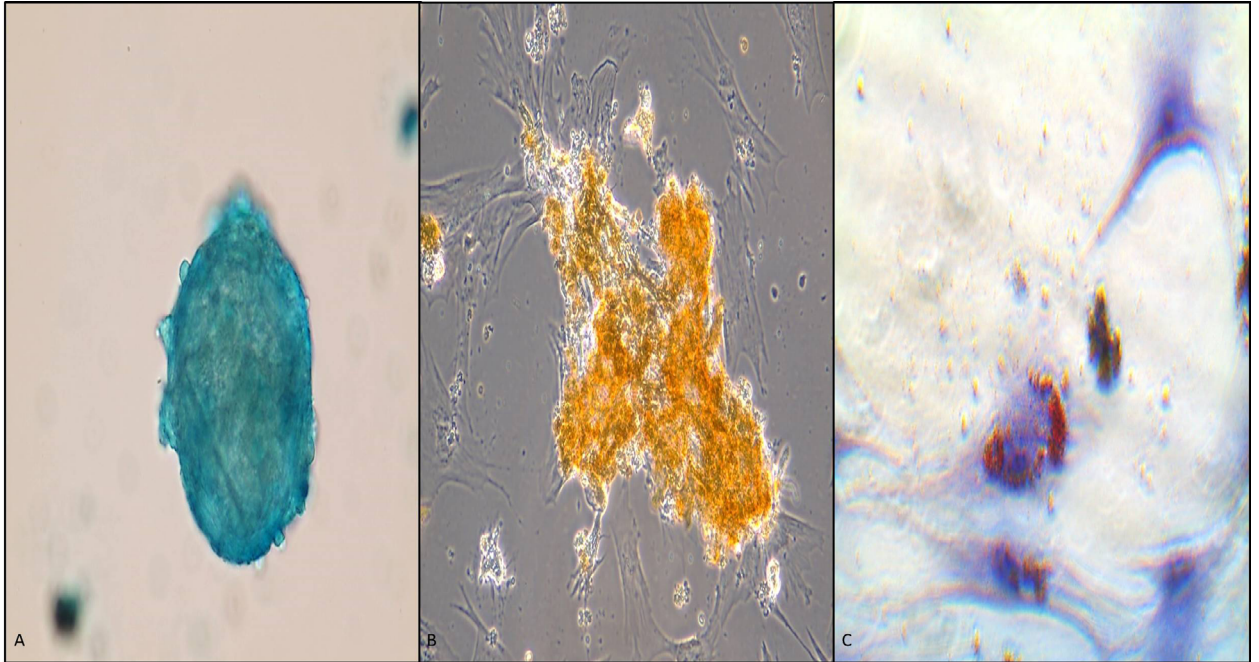


Figure 3. Chondrogenic differentiation (A), osteogenic differentiation (B), and adipogenic differentiation (C).

3. Conclusion

Both the ease of isolation of adipose tissue-derived stem cells and the possibility of using allogenic adipose tissue-derived stem cells in clinical cell-based treatments will contribute to advances in future in vitro studies on adipocytes and increase the knowledge available on adipogenic differentiation. Preclinical safety assessment

studies need to be conducted for the use of allogenic adipose stem cells in clinical cell therapies. The sorting of mesenchymal stem cells on the basis of gene expression will bring about radical changes in conventional methods and thereby contribute to a wide array of fields, from basic sciences to clinical treatment.

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