Qualitative and quantitative analysis of rabbit's fat mesenchymal stem cells[1]

Análise quantitativa e qualitativa de células tronco mesênquimais da gordura de coelhos

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ABSTRACT

Purpose: To present an experimental model of qualitative and quantitative analysis of mesenchymal stem cells from fat of rabbits obtained by lipectomy. The fat could be a great source for obtaining mesenchymal stem cells and to create conditions for repairing injured tissues by bioengineering. **Methods**: New Zealand rabbits (n= 10) adipose panicle (2-3 cm) were removed by lipectomy, fragmented and washed with PBS and enzymatically dissociated with trypsin/EDTA. Lately, these cells were incubated in culture medium DMEM and after 20 days, was performed quantitative analysis of the accession of first and second mesenchymal cells in cell culture bottles. **Results**: The fat total cells (CTF) were 1.62 x10^6 cells/mL and presented 98% of viability. These cells were taken for cultivation and after 20 days were counted 2.88 x10^6 cells/mL MSC. The same was done and after 20 days we quantified 4.28 x10^6 cells/mL MSC. **Conclusion**: The lipectomy of adipose panicule is a very satisfactory method to extract stem cells from fat, quantitatively and qualitatively.
Key words: Lipectomy. Stem Cells. Fats. Rabbits.

RESUMO

Objetivo: Apresentar um modelo experimental de análise qualitativa e quantitativa de células tronco mesênquimais proveniente da gordura de coelhos obtido por lipectomia. A gordura poderia ser uma grande fonte de obtenção de células tronco mesenquimais, criando condições para a reparação de tecidos lesados. **Métodos**: Foram removidos os panículos adiposos (2-3 cm) da região cervical de Coelhos Nova Zelândia (n = 10) por lipectomia. Os panículos foram fragmentados e lavados com PBS e, posteriormente, dissociados enzimaticamente com tripsina / EDTA. As células extraídas do panículo adiposo foram incubadas em meio de cultura DMEM e após 20 dias, foi realizada uma análise quantitativa da adesão de primeira e segunda passagem das células mesênquimais em garrafas de cultura. **Resultados**: Foram extraídas 1,62 x106 cel/ mL células totais de gordura (CTG) with 98% de viabilidade. Essas células foram levadas para o cultivo e após 20 dias, foi realizada a primeira passagem (1pd) sendo quantificadas 2,88 x10^6 cel/mL células tronco mesênquimais (CTM). Na segunda passagem (2pd) foi obtido 4,28 x10^6 cel/mL CTM. **Conclusão**: A lipectomia do paniculo adiposo é um método muito satisfatório para extrair células tronco a partir de gordura, quantitativamente e qualitativamente.
Descritores: Lipectomia. Células-Tronco. Gorduras. Coelhos.

[1] Research performed at Stem Cell Research Institute - IPCTRON and Otorhinolaryngology Advance Science Institute - ICAO, Sao Paulo, Brazil.

Introduction

Scientific and medical experts have the hope to promoting the legendary concept of regenerative medicine to develop therapies restoring injured tissues and acting on the nervous system lesions central[1,2]. This hope becomes more real each day with the use of advanced technologies such as cell culture, cell therapy, gene therapy and bioengineering, and the adult stem cells (ASC) source of these therapies.

The ASC defined as cells "wild" have the ability to self-renew and to differentiate into specialized cells[3] (Figure 1).

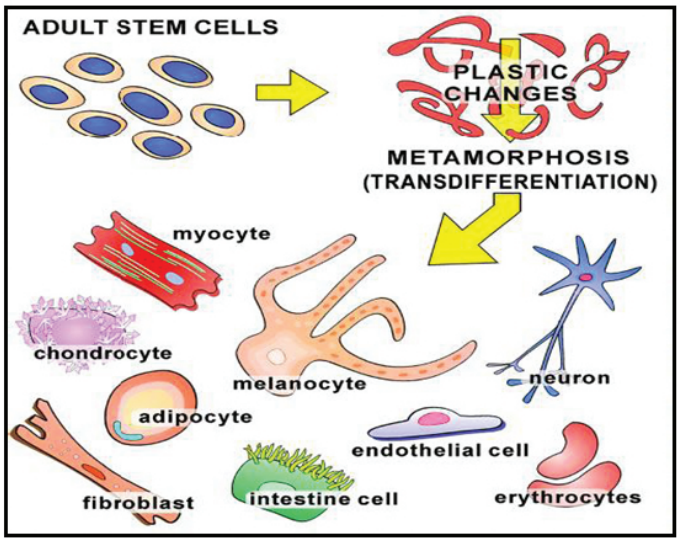

FIGURE 1 - The pluripotential ASC. (Modified from Leri *et al*.[13])

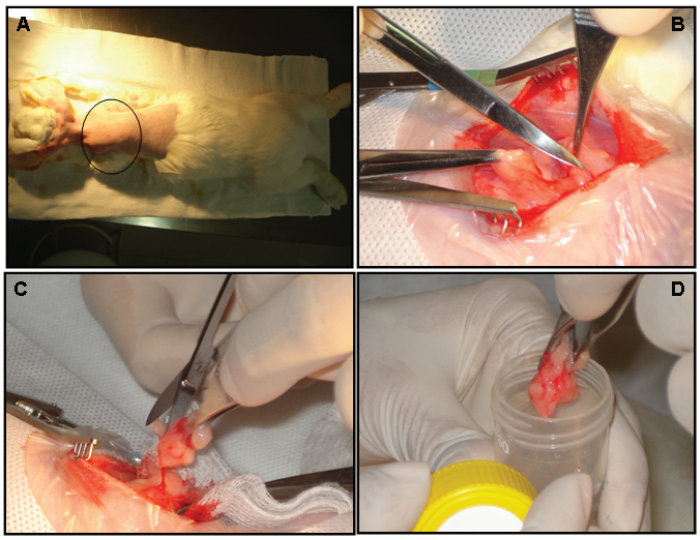

FIGURE 2 - Figures representing. **A**. dorsal region of rabbit; **B**. incision of rabbit dorsal region exposing the fat; **C**. collecting fat from dorsal region; **D**. Fat on tube with solution of phosphate-buffered saline (PBS)

These cells can be found in various niches such as bone marrow, peripheral blood, umbilical cord blood and placental (SCUP), dental pulp, hair follicle and fat, the latter objective of this study. The ASC are divided into two groups: mesenchymal stem cells (MSC) and hematopoietic stem cells (HSC).

MSC found in the bone marrow, also called stromal stem cells, represent 0.01% (10^{-2}) to 0.001% (10^{-3}) of nucleated bone marrow cells. These cells have great capacity for renewal and can differentiate into connective tissue cells such as osteoblasts, condroblasts, adipocytes, fibroblasts and myoblasts[3,4].

The HSC are derived from mesoderm[5]. The hematopoietic progenitors accumulate around the blood islands initially, migrating to the fetal liver reaching the bone marrow[6,7].

In the past 20 years, therapy with ASC of umbilical cord has been used successfully in patients with deficiency in bone marrow. The umbilical cord blood offer advantages over the bone marrow, it does not require a perfect compatibility with human leukocyte antigen (HLA). Moreover, this tissue shows a lower incidence of rejection, can be used allogeneic[8]. However, to obtain ASC of umbilical cord becomes limited, as can be gained only after the birth of the individual.

In this sense it is necessary to obtain ASC alternative sources, such as the adipose tissue presented in this study[9-12]. Therefore, the objective of this study was to perform a qualitative and quantitative analysis of mesenchymal stem cells extracted from fat tissue of rabbits by lipectomy.

Methods

This study was approved by the Research Ethics Committee of the Federal University of Sao Paulo (UNIFESP). New Zealand rabbits were used (n=10), 3 months, weighing approximately 3.5 kg from the animal colony of the Institute of Applied Sciences in Otorhinolaryngology (ICAO), Sao Paulo.

Collection of adipose tissue

The animals were submitted to general anesthesia with ketamine and zoletil (0.4 ml/kg each), by intramuscular injection. Subsequently, a longitudinal incision was performed 2 to 3 cm in the dorso-medial line, where is located the adipose panicle, in which the removed fat desired (lipectomy) (Figure 2).

Separation, cultivation and quantification of ASCs

After collection, the adipose tissue was fragmented (explant technique) into small pieces (about 1 cm) and washed with PBS supplemented with penicillin and streptomycin (1%) (Figure 3). Subsequently, the fragments were enzymatically dissociated with trypsin / EDTA. After the enzymatic process the material was subjected to centrifugation to the rupture of adipocytes to obtain a button of blood cells from the total fat (CTF) which were counted in a Neubauer chamber. Then were the qualitative part of the study through the cell viability using the dye trypan blue, which is associated to DNA when cell membrane rupture. After confirmation of cell viability, these cells were incubated in culture medium DMEM (high glucose, supplemented with fetal bovine serum and 10%) at 37°C with 5% CO_2. After 20 days, was performed quantitative analysis of the accession of mesenchymal cells (MSC 1pd) in cell culture bottles.

By the enzymatic method (trypsin + EDTA) performed the resuspension of MSC to wrap them in two bottles of the second culture passage (MSC 2pd), and again after 20 days quantify them in a Neubauer chamber.

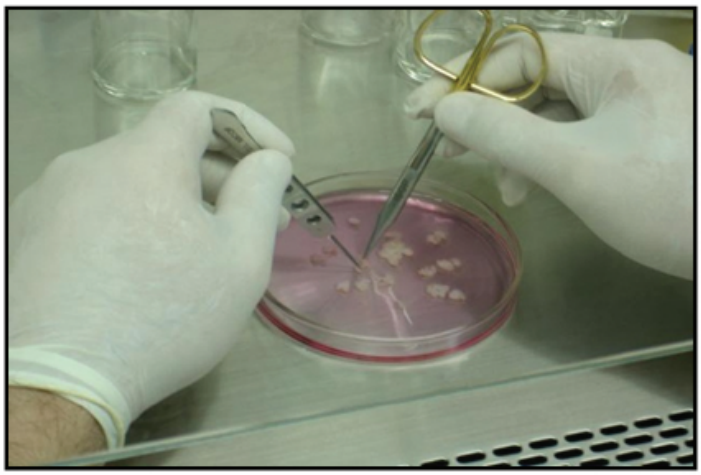

FIGURE 3 - Technique of explants of adipose tissue extracted by lipectomy

Results

Quantitative analysis of blood cells of total fat (CTF) before and after culture MSCs

After extraction of CTF, the counting was done in Neubauer chamber which were 1.62 ± 0.17 (x10^6 cel/mL) CTF. These cells were taken for culture and after 20 days were counted 2.88 ± 0.24 (x10^6 cel/mL) MSC (1 pd). These cells were resuspended with trypsin and placed in two bottles which were kept in culture for another 20 days and counted again, quantified 4.28 ± 0.36 (x10^6 cel/mL) MSC (2 pd), as shown in Figure 4.

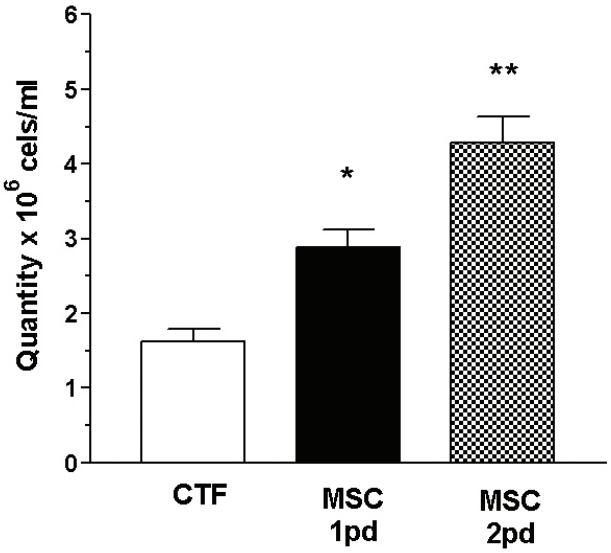

FIGURE 4 - Histogram representing the total cells extracted from adipose panicle rabbit (CTF) and cultivated mesenchymal stem cells after first passage (MSC - 1pd) and second passage (MSC - 2pd). The columns and bars respectively indicate mean ± standard error of mean of cells from 10 rabbits. Statistically different from the CTF, * 0.001 and ** p ≤ p ≤ 0.0001 (Student t-test).

Qualitative analysis of mesenchymal stem cells from adipose tissue (MSC)

Cell viability

The CTF (Figure 5) and MSC (data not show) cell viability was done by the reaction with Trypan Blue showing a viability of 98%.

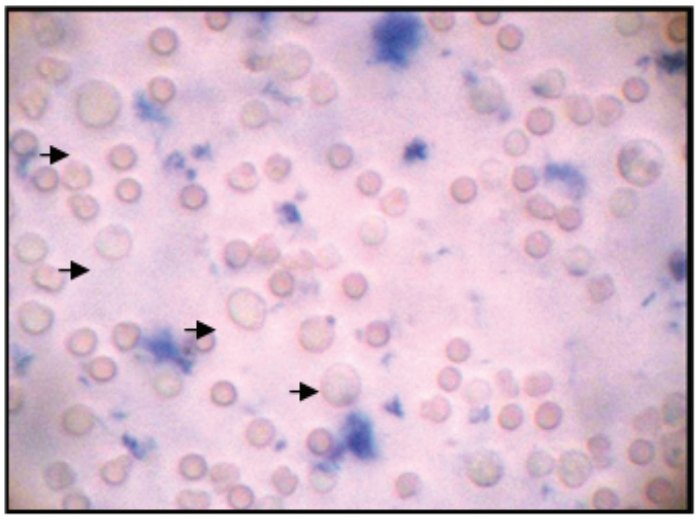

FIGURE 5 - Figures represent the cell viability of CTF with trypan Blue. The arrows represent CTF who are colorless.

Discussion

This study showed that the enzymatic treatment of rabbit adipose panicle with Trypsin / EDTA resulted in obtaining a large number of blood cells of total fat (CTF). Unfortunately these cells can not be characterized as mesenchymal and hematopoietic cells by using optical microscopy. Because of the population of cells extracted of adipose panicule observed in microscope show appearance of mononuclear and granular cells, we called them by blood cells from total fat (CTF). This work has shown that we can extract 1.62 x10^6 cells/mL of CTF from adipose panicule with good viability. In the same way Forriol and Esparza[14] suggest that fat we can extract 4x10^7 cell/mL of CTF.

Despite we have obtained a good number of CTF, we can also amplify these cells using a culture method. These cells, when maintained in culture in an appropriate medium, were amplified by 77% (on relation of CTF) showing a good option to apply these cells to repair injuries. Because of a few studies of fat stem cells, we can not compare exactly the proliferation of these cells types. However, other authors have amplified by about 40% the blood cells extracted from bone marrow when these cells were maintained on culture[14-16].

The cells on culture were also show to be able to adhere on culture bottle, extending and converging like a tissue, forming a fibroblastoid colony (CFU-F), like other authors have observed[13-16].

Is important to note that the second passage also increase the quantity of MSC by 164% (on relation of CTF) showing that these cells have a good capacity of proliferation, like other authors have observed[20-24]. These authors have also observed that these cells maintain the same physiology until five passages[23,24].

Conclusion

The fat can be an excellent alternative way to obtain stem cells with good viability. These cells have greatly increased its population even forming a colony. The fact of forming a colony suggests that these cells have the capacity to form tissues and organs.

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