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Mesenchymal Stem Cells - Emphasis in Adipose Tissue

Bruno Machado Bertassoli[1*], Antonio Chaves de Assis Neto[2], Franceliusa Delys de Oliveira[2], Maria Angélica Machado Arroyo[2], Juliana Shimara Pires Ferrão[2], Jodonai Barbosa da Silva[2], Graciela Conceição Pignatari[2] and Patrícia Beltrão Braga[2]

[1]Instituto de Ciências Biológicas; Departamento de Morfologia PPG em Biologia Celular; Universidade Federal de Minas Gerais; Av. Presidente Antônio Carlos; 31270-901; Belo Horizonte - MG – Brasil. [2]Faculdade de Medicina Veterinária e Zootecnia; Departamento de Cirurgia; PPG em Anatomia dos Animais Domésticos e Silvestres; Universidade de São Paulo; Av. Professor Dr. Orlando Marques de Paiva; 05508-270; São Paulo - SP - Brasil

ABSTRACT

The study of stem cells has evolved rapidly in recent decades. The importance is given to the concept that these cells are potentially able to become any cell type and have the power of self-renewal throughout the life of the organism. Mesenchymal stem cells (MSCs) can be isolated from various organs of the body such as bone marrow, adipose tissue, synovium, muscle and dermis, deciduous teeth, umbilical cord, placenta, liver, spleen and thymus. After their isolation in vitro*, mesenchymal stem cells have the capacity to differentiate into various mesenchymal lineages and various tissues after the use of appropriate cultures. Studies have reported that mesenchymal stem cells from adipose tissue have the potential to differentiate themselves, like the cells commonly studied bone marrow. Adipose tissue is attractive due to its easy access, rapid expansion* in vitro *and only one collects the large amount of tissue. This review intends to show the protocols for isolation, cell culture and means of commercial cellular differentiation most widely used with emphasis on adipose tissue.*

Key words: Adipose Tissue, Stem Cells, Mesenchymal Stem Cells

INTRODUCTION

The study of stem cells has evolved rapidly over the last decades. The importance is given to the concept that these cells are potentially able to become any cell type and undifferentiated cells are capable to differentiate into progenitors and have the power of self-renewal throughout life of the organism (Schwindt et al. 2005). These characteristics are used to classify the stem cells in totipotent, pluripotent, multipotent, oligopotentes, or omnipotent (Schwindt et al. 2005; Oliveira et al. 2006; Spangrude et al. 1988). The concept and practice of transplanting stem cells begins with the hematopoietic system, in which a hematopoietic stem cell gives rise to all blood lineages from a single cell (Pereira 2008), comprising 0.05 to 0.1% of human bone marrow and circulating hematopoietic cells. An interesting aspect of marrow stem cells is their plasticity, its ability to convert from one cell type to another, and differentiate into not-hematopoietic cells (Grotto and Noronha 2003).

The stem cells can be of different origins: embryonic, fetal and adult. Mesenchymal stem cells (MSCs) from the adult human are multipotent cells and are able to differentiate into osteocytes, chondrocytes, adipocytes and cell lines when stimulated under appropriate conditions (Jaiswal et al. 2000; Zuk et al. 2002). Thus, theoretically,

*Author for correspondence: brunobertassoli@gmail.com

these cells could be multiplied in the laboratory and induced to form specific cell types (Pereira 2008).

CONCEPTS OF MESENCHYMAL STEM CELL (MSCs)

The cells that are found in bone marrow stroma, called mesenchymal stem cells (MSCs), are non-hematopoietic multipotent stem cells that show a capacity, when under an enabling environment, to renew and differentiate into various cell lines of mesodermal origin including: bone cell, muscle and chondrogenic cells (Dicker et al. 2005; Sensebé et al. 2010). The MSCs were first studied in 1970 and it was observed that some cells were well adhered to culture plates. The morphology of these cells was very similar to that of fibroblasts *in vitro* and that cells were clustered and formed some colonies (Johnstone 2002). These cells were derived from mesodermal progenitors, but recently it was discovered that they also originated from the neuro-epithelium of the embryo, as observed by the fact the they expressed the marker Sox-1 (Takashima et al. 2007). It is not yet confirmed whether the source of all the MSCs is mesodermal progenitors (Sensebé et al. 2010).

The first place where these stem cells were found was in the vascular bone marrow stroma, located in all the tissues. However, MSCs have also been observed inside the wall of blood vessels (Crisan et al. 2008). Besides the bone marrow, there are MSCs that are in peripheral blood and are also in the umbilical cord, but when compared to the amount of cells found in these sites with those found in bone marrow, bone marrow becomes more interesting by having a larger amount of MSCs, although the growing conditions for these cells are not well-established (Wexler et al. 2003; Schwindt et al. 2005). According to the International Society of Cellular Therapy, the MSCs, to be classified as such, must have certain important features: ability to adhere to plastic, to express specific membrane molecular markers such as CD73, CD90 and CD105, do not express CD14, CD34 and CD45 markers, and have the ability to differentiate into three different cell types (osteogenic, adipogenic and crondrogenic). With these characteristics, they could be called mesenchymal stem cells (Ugarti et al. 2003). With all these features, they arouse great interest in research aimed on regenerative medicine (Jiang et al. 2000; Bianco et al. 2002). The MSCs have been collected from different adult tissues and studies have shown that when these cells were studied separately, there were significant differences between them, according to their origin (Puissant et al. 2005; Bochev et al. 2008).

Mesenchymal stem cells (MSCs) are found in the perivascular adipose tissue, called mesenchymal stem cells derived from adipose tissue. These cells have the same genes and the same differentiation potential of mesenchymal cells from other locals (Lin et al. 2008). Adipose tissue has been an important source of adult stem cells due to worldwide increasing levels of obesity, making it easier to obtain this tissue (Gimble et al. 2007). The great advantage of these mesenchymal cells is their abundance, availability and accessibility, when compared to other sources of MSCs (Zuk et al. 2001). Behaving like stem cells, adipocytes have the multipotent capacity to undergo chondrogenic, osteogenic, cardiogenic, neurogenic and angiogenic differentiation (Poulos et al. 2010).

DIFERENTIATION OF MSCs

After their isolation *in vitro*, MSCs show a capacity to differentiate into various mesenchymal lineages (Table 1) (Lee et al. 2010) and in various tissues when induced after the use of appropriate cultures. The MSCs can differentiate into osteoblasts, adipocytes, chondrocytes (Ringe et al. 2002; Kumar et al. 2007), cardiomyocytes and support stromal cells from hematopoiesis (Pittenger et al. 1999; Caplan 2005). Its plasticity, however, is not restricted to mesenchymal derivatives. Reports suggest that MSCs can also differentiate into neurons (Moorman et al. 1999). The expansion of MSCs from different sources (bone marrow cells, peripheral blood, embryonic annexes, among others) has been widely studied due to their ability to differentiate into several lineages, and represent a promising population for cell-based therapies in veterinary medicine. There is still little information about the origins of MSCs, but there is a theory that it may be from neuroectodermal origin, from neuroepithelial cells (Takashinma et al. 2007).

Table 1 - Origin and cell types derived from mesenchymal stem cells.

Isolation	Mesenchymal Stem Cells – Differentiation
Bone Marrow	Osteoblasts
Trabecular Bone	Chondrocytes
Periosteum	Adipocytes
Articular Cartilage	Cardiac Myocites
Synovium	Fibroblasts
Synovial Fluid	Myofbroblasts
Muscles	Skeletal Myocites
Adipose Tissue	Tenocytes
Tendon	Retinal Cells
Blood	Neural Cells
Blood Vessels	Astrocytes
Umbilical Cord	Hepatocytes
Skin	Stroma Support
Spleen and Thymus	Pancreact Cells

(Source: ADPT of Pountos and Giannoudis 2005)

These MSCs can be isolated from various organs of the body such as bone marrow, adipose tissue, synovial membrane, muscle, dermis, deciduous teeth, umbilical cord, placenta, liver, spleen and thymus (Caplan 2005; Meirelles et al. 2006). The population of stem cells derived from adipose tissue digested with collagenase, also called stromal vascular fraction, is able to differentiate into several cell lineages, including adipose tissue, cartilage, bone, skeletal muscle, neuronal cells, endothelial cells, cardiomyocytes and smooth muscle cells (Ferrari et al. 1998; Pittenger et al. 1999; Romanov et al. 2003; Baptista et al. 2009).

Adipose tissue represents the ideal source of autologous stem cells, since its acquisition is easy, with minimal discomfort for the patient and often with greater proliferative capacity than the cells derived from bone marrow. This characteristic may be intrinsic to the cells, or a result of increased density of stem cells in the initial population. Thus, there is less invasiveness and complexity in the collection, which results in less suffering for the patient, associated with the ability to recover significant numbers of mesenchymal cells, sufficient to prevent extensive expansion in the culture to generate effective clinical potential of cells derived from adipose tissue in relation to other methodologies (Yokomizo et al. 2011). MSCs show promising results in preclinical studies and clinical trials in heart disease, lung, spinal cord and central nervous system injuries, and bone defects in cartilage (Nardi and Meirelles. 2006; Le Blank 2006). The bioengineered of tissue in combination with biomaterials has shown satisfactory results with the use of MSCs (Zhang et al. 2007).

ISOLATION AND CULTURE OF MESENCHYMAL STEM CELLS – ADIPOSE TISSUE

The most commonly method used for the isolation of MSCs from liposuction was published by Zuk et al. (2001) that described the processes of aspiration of adipose tissue, digestion of the tissue, cultivation and characterization of mesenchymal cells. When the cells are isolated from the donor tissue, they can be kept in various forms. A small piece of tissue that adheres to a surface of growth, either spontaneously, or by mechanical aid, like blood clots, or an extracellular matrix constituent of the collagen, generally gives support for the cell's growth. This kind of culture is known as a primary explant and the cells that migrate from the tissue are growing cells. These are selected in the first instance due to their ability to migrate from the explant and subsequently, if sub-cultured, the ability of proliferation. When a sample of tissue is disaggregated, either mechanically, or enzymatically, a suspension of cells and small aggregates capable of binding to a solid substrate is formed, forming then a monolayer. The cells monolayer with the capacity to proliferate is selected from the first subculture, and like the primary explant, it can possibly originate a stem

cell line. The disaggregated tissue is capable of producing large crops faster than explant culture, but the latter may be preferred when small fragments are obtained, or when tissue frailty prevents the cell's survival after disaggregated (Freshney 2006).

The cultivation of MSCs is performed by selecting the cells with adhesiveness to plastic, whereas cells that remain in suspension are easily removed. The other "contaminants" cell type such as macrophages, are removed after a certain number of passages in culture (Javazon et al. 2004). In cultures and in suitable conditions of cultivation, the MSCs show fibroblastoid format, adhesion to plastic substrate, self-renewal and differentiation into cell types, including bone, cartilage, adipose tissue, tendons and muscles (Mendelow et al. 1980; Pittenger et al. 1999; Pountos and Giannoudis 2005; Nardi and Meirelles 2006). They can be expanded by more than 40 generations keeping multipotent capacity while reducing the rates of mitosis, and there is a high probability of accumulation of mutations, making it inadvisable to its clinical use in these circumstances (Deans and Moseley 2000). Another characteristic of MSCs is that when grown in low-density, the adhesion and colony formation is fast. It is assumed that these colonies are derived from a single precursor cell (Deans and Moseley 2000). However, even after years of studies on the composition of cell cultures, the selection of these cells still remains empirical. Some cell cultures are commercially available and are listed in Table 2.

Table 2 - Principal available means to grow mesenchymal stem cells.

Trade Name	Description
Basal Medium Eagle (BME)	Containing only essential amino acids. It needs to be supplemented with fetal calf serum to 10%.
Minimum Essential Medium Eagle (MEM)	Contains more amino acids and in higher concentration than BME. Requires supplementation with fetal calf serum to 10%.
Medium MEM modified Dulbecco (DMEM)	Contains four times the concentration of amino acids and vitamins that the BME.
Medium DMEM modified Iscove (IMDM)	Complete formulation that includes bovine albumin, transferrin, selenium, and others. Requires supplementation with fetal calf serum to 10%.
Medium F-10 Ham	It can be supplemented with proteins and hormones. Contains metals such as iron, copper and zinc.
Medium F-12 Ham	Provides protein supplementation for cells. It may have its use combined with IMDM.

Adipose tissue is derived from the embryonic mesenchyme and contains a stroma that is easily isolated. Some studies have shown that there is a potential stem cell compartment in adipose stroma. This cell population, called processed lipoaspirate (PLA), can differentiate into osteogenic, adipogenic, myogenic and chondrogenic progenitor cells. However, the most appropriate process for obtaining the aspirate and the number of viable cells in tissues are not well established (Pereira et al. 2008).

INDUCTION OF ADIPOSE TISSUE

According to Ren et al. (2011), adipose tissue is abundant, accessible and easy to obtain from the body, increasing the interest in adipose-derived stem cells (ADSCs) for tissue engineering. They are expected to become seed cells for improving tendon healing such as tissue engineering, cell therapy and gene therapy. ADSCs have been obtained from humans, mice, rats, and bovines, but few studies have used goats.

GOAT AND RAT

A goat adipose-derived stem cell (ADSC) line was established and compared to a rat-line. Goat ADSC had normal diploid cells after subculture. Proliferation of goat ADSCs was faster than rat cells in the same conditions. Adipogenic induction resulting in lipid droplets and peroxisome proliferator-activated receptor (PPARc2) expression were observed. For adipogenic differentiation and confirmation, cells were incubated with DMEM/F12 containing 3% FBS,

antibiotics, 33 lM biotin, 17 lM pantothenic acid, 1 lM insulin, 1 lM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 5 lM rosiglitazone and 5% rabbit serum for three days, then fed inducing medium without rosiglitazone and IBMX. Control cultures were fed only with DMEM/F12 containing 10% FBS and antibiotics (common ADSCs medium). After 21 days of culture, cells were fixed with 10% formalin and incubated for 20 minutes with Oil Red-O to visualize lipid droplets. Adipogenic differentiation was confirmed by Nile Red staining of lipid droplets. The adipogenic determination gene PPARc2 was detected by PCR. Adipogenic differentiation of goat and rat ADSCs was confirmed by Oil Red-O staining. After feeding ADSCs with adipogenic-inducing medium for 21 days, oil droplets were present in the cytoplasm (Fig. 1). The oil droplets appeared to be larger in goat cells than in rat cells and the droplets had a rounder shape (measure). The expression of peroxisome proliferator-activated receptor (PPARc2) was seen in both goat and rat adipogenic-induced cells (Fig. 1), indicating that both types of ADSC cells had differentiated into fat cells following adipogenic induction (Ren et al. 2011).

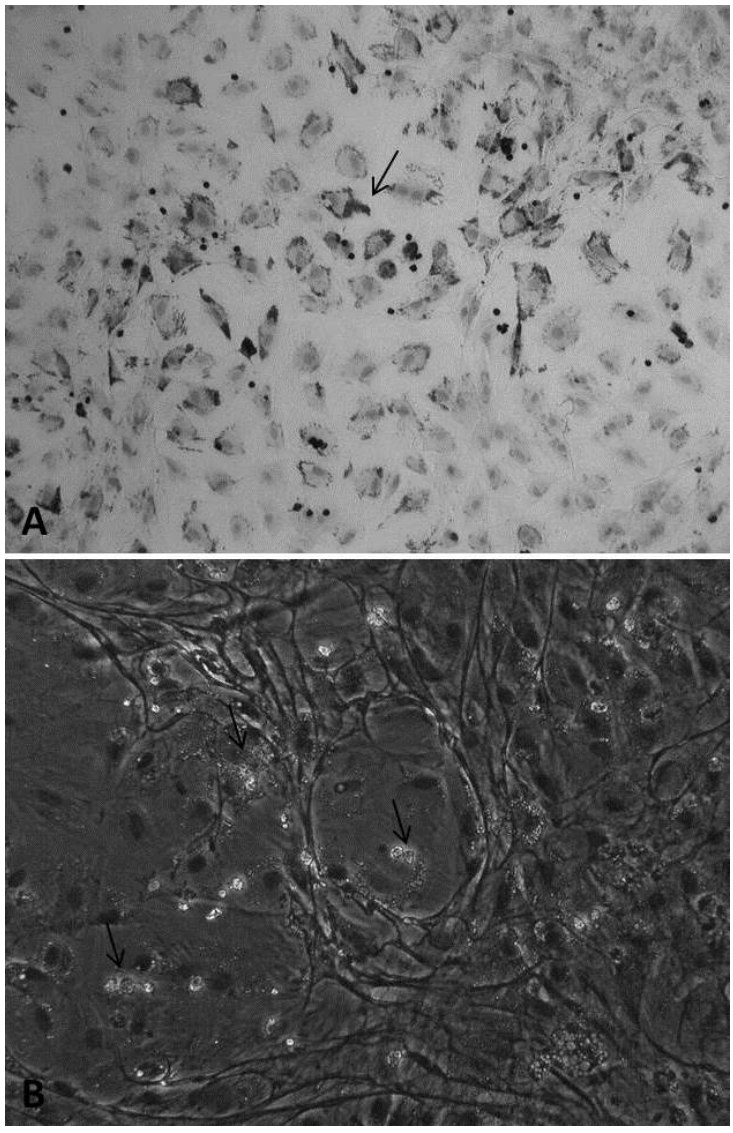

Figure 1 - Adipogenic differentiation of rat ADSCs. ADSCs treated with adipogenic media for 21 d stained with Oil Red-O. Arrows show oil droplets (original magnification _ 40).

CHICKEN

According to Khuong and Jeongin (2011), chicken epithelial oviduct cells (COCs) are part of important supportive tissues in chicken reproductive organs responsible for secretion of the majority of chicken egg protein. In chickens, the biological process of adipocyte differentiation has been extensively studied *in vitro* using a number of cell types, including a preadipocyte precursor cell line, a number of other undifferentiated cell lines, and chicken embryonic fibroblasts. However, adipogenic differentiation in epithelial cells has not yet been achieved. In their study, they induced COCs to differentiate into adipocytes using chicken serum at concentrations of 5 and 10%. After a 24 h culture period at 37°C in a humidified 5% CO_2 atmosphere, oviduct cell morphology changed dramatically through the formation of lipid droplets, observed by Oil Red-O staining. Also, chicken serum strongly induced 3T3-L1 preadipocyte cell differentiation into adipocyte. In addition, mRNA expressed levels of peroxisome proliferator-activated receptor gamma, adipocyte fatty acid-binding protein (aP2), and CCAAT enhancer-binding protein alpha were significantly increased 48 h after induction. These results suggested that COCs could be induced to differentiate into adipocyte-like cells. Moreover, through this study, they confirmed that chicken serum was an effective adipocyte differentiation-inducing agent.

RABBIT

Stem cells obtained from the adipose tissue of rabbits are seeded in culture dishes of 25 ml volume at a rate of 2.5 x 10^5 cells per plate and cultured in DMEM containing 10% fetal bovine serum supplemented with 62 µg/ml penicillin and 50 µg/ml streptomycin. From the first 48 h, the medium is changed every two days, the first two exchanges being mediated by a PBS washing to remove any residue of erythrocytes, or adipose tissue that could have left over in the plaque. These exchanges continue until the cells reach approximately 70% confluence, when they are subjected to trypsinization (Pereira et al. 2008).

CANINE, EQUINE AND PORCINE

Schwarz et al. (2011) mentioned adult stem cells were of particular interest for therapeutic use in the field of regenerative medicine. ADSCs are an attractive stem cell source for all the fields of regenerative medicine because adipose tissue – and therewith cells – can easily be harvested from each donor. However, common expansion using fetal bovine serum (FBS) cannot be used for clinical applications and xenogenic proteins must be avoided. Adipose tissue from equine, canine and porcine donors was digested with collagenase to isolate the ASCs. The ASCs were either expanded in a cell culture medium supplemented with FBS, or in a serum-free medium (UltraCulture; UC), supplemented with a serum substitute (UltroserG). From all three animal species, the adipogenic and osteogenic differentiation potential of ASCs cultured with different media was analyzed *in vitro*. The cell proliferation analysis showed a population doubling time of 48-68 h for canine cells, 54-65 h for porcine cells and 54-70 h for equine cells, expanded in different media. Except for porcine ASCs, cells cultured in the media supplemented with FBS grew faster than the cells expanded in UC medium with UltroserG. Yet, all the cells maintained their potential to differentiate into adipocytes and osteoblasts.

UltraCulture medium containing UltroserG can for all examined species be recommended if FBS needs to be avoided in the expansion of donor-derived (stem) cells. Canine ASCs were cultured either in Dulbecco's modified eagle medium (DMEM) high glucose (PAA Laboratories GmbH, Pasching, Austria), containing 10% FBS (fetal bovine serum) and 1% penicillin–streptomycin (PAA Laboratories GmbH, Pasching, Austria), or in a serum-free medium (UltraCulture, Cambrex, USA), supplemented with a serum substitute (2% UltroserG, Pall Biosepra, France), 2 mM L-glutamine (Invitrogen, Darmstadt, Germany) and 1% penicillin–streptomycin. Porcine ASCs were cultured in the same manner as described for canine cells with minor modifications, using an Alpha-modified eagle medium (a-MEM) instead of DMEM medium. Equine ASCs were cultured by the same method as described for the canine cells, in DMEM medium compared to UC medium. Additionally, a third medium was tested: DMEM high glucose, containing 10% autologous serum and 1% penicillin–streptomycin. All the cells were maintained in a humidified atmosphere of 95% air with 5% carbon dioxide at 37'C. After 72 h, the medium was exchanged and thereby non-adherent cells were washed away. During the

following cultivation period, cells were washed with PBS and subsequently detached by incubation with 1% trypsin after reaching 80% confluence. Cells were counted using a hemocytometer and re-seeded in new culture dishes. The medium induction consisted of DMEM containing 10% serum with isobutylmethylxanthine (IBMX) (1 mM), dexamethasone (1 lM), indomethacin (0,2 mM), penicillin/streptomycin (40 IU/ml) and insulin (0,1 mg/mL). The medium conservation consisted of DMEM with 10% serum, penicillin/streptomycin (40 IU/ml) and insulin (0.1 mg/mL). Differentiation was achieved by culturing in the medium induction for five days, followed by medium conservation for two days. This sequence was repeated twice. The adipogenic differentiation medium for porcine ASCs was DMEM with 10% serum, penicillin/ streptomycin (40 IU/ml), indomethacin (0.1 mM), dexamethasone (1 lM), isobutylmethylxanthine (IBMX) (0,05 mM) and Insulin-Transferrin-Selenium (ITS) (1%). Equine samples were induced to differentiate with autologous serum instead of FBS. Subsequently, lipid droplet formation was assessed with Oil Red-O staining (Fig. 2).

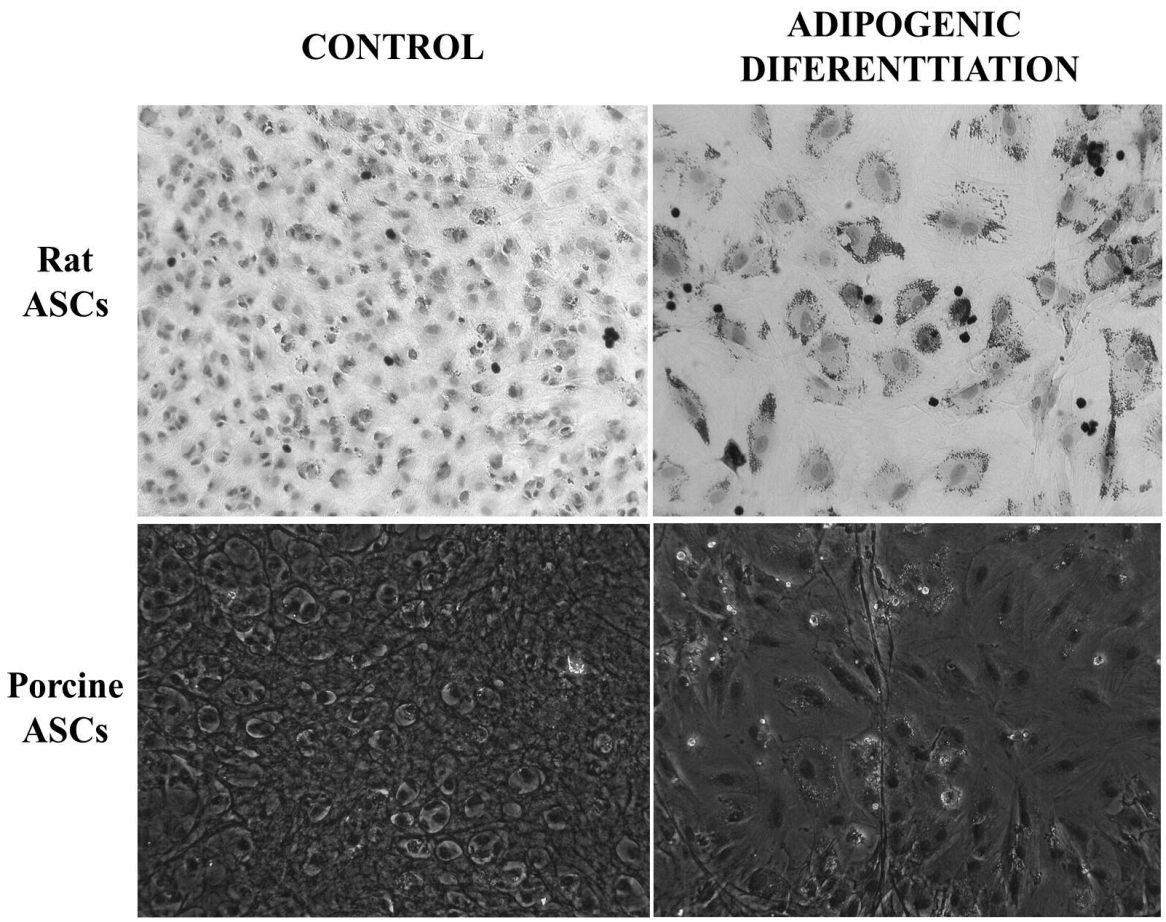

Figure 2 - Differentiation potential is shown with a representative picture of one donor of each species. Osteogenic differentiation was assessed with alizarin red staining and adipogenic differentiation was proven with Oil Red O staining at d 21 of the cultivation period. Control wells were treated with the same medium as the stimulated cells with the exception of osteogenic/adipogenic differentiation factors (20_ objective).

MOUSE

Sellayah et al. (2011) demonstrated that orexin (OX) neuropeptides stimulated the feeding and arousal. The deficiency of orexin is implicated in narcolepsy, a disease associated with the obesity, paradoxically facing the reduced food intake. They showed that obesity in orexin-null mice was

associated with the impaired brown adipose tissue (BAT) thermogenesis. Failure of thermogenesis in OX-null mice was due to inability of brown preadipocytes to differentiate. The differentiation defect in OX-null neonates was circumvented by OX injections to OX-null dams. *In vitro,* OX triggered the full differentiation program in mesenchymal progenitor stem cells, embryonic fibroblasts and brown preadipocytes via p38 mitogen activated protein (MAP) kinase and bone morphogenetic protein receptor-1a (BMPR1A)-dependent Smad1/5 signaling. Their study suggested that obesity associated with OX depletion was linked to brown-fat hypoactivity, which led to dampening of energy expenditure. Thus, orexin plays an integral role in adaptive thermogenesis and body weight regulation via effects on BAT differentiation and function.

HUMANS

According to Sorrell et al. (2011), there is an increasing demand for soft-tissue substitutes in plastic and reconstructive surgeries to replace autologous adipose tissue grafts for trauma, or tumor resections. Adipose stromal cells were seeded at a density of 35,000 cells/cm^2 onto either 24-well plates, or 6-well culture plates; the 24-well plates were used for immunohistochemical studies and Oil Red-O staining and the 6-well plates were used to create the material for histological sectioning. These cells were cultured for one week in EGM-2 MV that was supplemented with 270 mM ascorbate 2-phosphate (Sigma Chemical Co.) Cellular populations derived from the adult human bone marrow and adult interstitial white adipose tissue contained multipotential cells that could be induced to differentiate down the adipocyte lineage.

According to Sarkanen (2012), the induction of adequate vascularization, a major challenge in tissue engineering, has been tried with numerous methods but with unsatisfactory results. Adipose tissue, an active endocrine organ with dense vasculature, secretes a wide number of angiogenic and adipogenic factors and seems an attractive source for these bioactive factors. They produced a novel cell-free extract from mature human adipose tissue (adipose tissue extract, ATE) and analyzed the ability of this extract to induce angiogenesis and adipogenesis *in vitro* and studied the cytokine and growth factor composition of ATE with ELISA and cytokine array. They demonstrated that ATE, when added as cell culture supplement, effectively induced triglyceride accumulation in human adipose stem cells at concentrations from 200 mg/mL upward in less than a week and caused elevated levels of adipocyte differentiation markers (proliferator-activated receptor gamma and acyl-CoA-binding protein) when treated with at least 350 mg/mL of ATE. ATE induced angiogenesis from 450 mg/mL upward after a week *in vitro*. ATE contained numerous angiogenic and adipogenic factors, for example, vascular endothelial growth factor, basic fibroblast growth factor, interleukin-6, adiponectin, angiogenin, leptin, and insulin-like growth factor-I, as well as lower levels of a wide variety of other cytokines. They present a novel cell-free angiogenesis- and adipogenesis-inducing agent that is cell-free and easy to produce, and its effect is dose dependent and its composition can be easily modified. Therefore, ATE is a promising novel agent to be used for angiogenesis induction to overcome the challenge of vascularization and for adipogenesis induction in a wide variety of tissue engineering applications *in vitro* and *in vivo*. ATE is also efficient for reproduction and modeling of natural adipogenesis *in vitro* for, for example, obesity and diabetes studies.

INFLAMMATION

According to Ye and Gimble (2011), adipose-derived stem cells (ASCs) are able to differentiate into multiple linages of progenitor cells for adipocytes, endothelial cells, fibroblasts and pericytes. Differentiation of ASCs into those progenitors is regulated by the adipose tissue microenvironment. As a major factor in the microenvironment, inflammation may favor ASC differentiation into the endothelial cells through induction of angiogenic factors. At the same time, inflammation inhibits ASC differentiation into adipocytes by suppressing PPARγ activity and insulin signaling pathway. In this context, inflammation may serve as a signal mediating the competition between adipocytes and endothelial cells for the limited source of ASC.

Differentiation of each linage of progenitor cells from ASC is modulated by the physical microenvironment within adipose tissue. In obesity, there may be a competition between these differentiation pathways for the limited number of ASCs. Inflammation and angiogenesis are two important microenvironmental factors that

determine the outcome in the competition for lineage commitment of progenitor cells. Inflammation may suppress the generation of pre-adipocytes from ASC by inhibition of adipocyte differentiation. In contrast, inflammation may promote differentiation of ASC into endothelial precursors. As a result, the dynamics of adipocyte turnover in adipose tissue is interrupted. This disbalance can be restored by the drugs such as TZDs, which induces the pre-adipocytes differentiation to adipocytes. Lack of endothelial cells impairs angiogenesis and leads to ATH, which triggers the compensatory inflammatory response. This engages a feedback response, whereby inflammation promotes ASC differentiation into endothelial cells to improve the blood supply in adipose tissue through angiogenesis. Although these possibilities are supported by much evidences, direct proof of the possibilities remains to be done in the adipose tissue in obesity.

DISCUSSION

Zhang et al. (2006) and Barry and Murphy (2004) stated that stem cells could be classified in embryo, or adult cells, depending on their origin and biological properties. The embryonic stem cells are derived from the inner layer of the blastocyst stage embryo and have the capability to origin several cell lineages. The adult stem cells are present in the organs, or tissues and are responsible for the maintenance of tissue integrity for tissue repair and reshuffle due to lesions. Jaiswal et al. (2000), Zuk et al. (2002) and Pereira (2008) reported that those cells were capable to differentiate into osteocytes, chondrocytes, adipocytes and cell lineages when stimulated under appropriate conditions. Valentini et al. (2010) defined the mesenchymal stem cells (MSCs) as multipotent cells able to differentiate into several cell lines *in vivo* and *in vitro* under controlled conditions, corroborating with Lee et al. (2010), who showed that these cells had already been differentiated into a diversity of cell types.

The stem cells (MSCs) can be isolated from multiple organs, for example, bone marrow, adipose tissue, synovial membrane, muscles, dermis, deciduous tooth, umbilical cord, placenta, liver, spleen and thymus (Meirelles et al. 2006). Schauwer et al. (2011) isolated the MSCs from peripheral blood and embryonic annexes. According to Lin et al. (2008), the stem cells originated from adipose tissue have the same genes and differentiation potential as mesenchymal from other sites. Poulos et al. (2010) demonstrated that adipocytes had the capability to undergo a condrogenic, osteogenic, cardiogenic, neurogenic and angiogenic differentiation.

In the adipose tissue, the presence of such cells was revealed by Zuk et al. (2002), who proved the existence of stem cells in human adipose tissue and compared the differentiation potential of these cells with the MSCs isolated from bone marrow. Fraser et al. (2008) and Ugarti et al. (2003) also showed that adipose derived from tissue stem cells were similar to those obtained from bone marrow, making them particularly attractive due to the fact to obtain them in large amounts in a single collection and rapid *in vitro* expansion. Javazon et al. (2004) made the culture of MSCs by sectioning the cells with adhesion properties to plastic and the cells that remained in the suspension were easily removed. Another way to select the MSCs is to analyze their morphology, which must be fibroblastoid (Nardi and Meirelles 2006). Reina (2003) demonstrated medium selection was empiric; other authors showed that each isolation source and species utilized require a different culture medium (Im et al. 2001; Huang et al. 2004).

CONCLUSION

The above descriptions showed that the mesenchymal stem cells (MSCs) have reparative potential and could be obtained, isolated and expanded in different ways and conditions, due to the diversity of the protocols of isolation and existing cultures. This diversity brings benefits related to the development of procedures that provide high performance and viability of mesenchymal cells, thus increasing the final number of cells transplanted. Adipose tissue has proved means of obtaining good cells, since its collection is little elusive and its plasticity is diversified. Advances in the studies with mesenchymal stem cells (MSCs) of adipose tissue in humans and veterinary medicine raises the need to increase the basic knowledge about their origin, acquisition and processing.

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