Adipose-derived stem cells (ADSC) in the viability of random skin flap in rats

Caio Vinicius Suartz¹, Silvana Gaiba², Jerônimo Pereira de França³, Antonio Carlos Aloise⁴, Lydia Masako Ferreira⁴

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¹Graduate Student, Department of Surgery Federal University of São Paulo, São Paulo, SP, Brazil. Technical procedures.
²PhD Plastic Surgery Division, Department of Surgery Federal University of Sao Paulo, São Paulo, SP, Brazil. Technical procedures, acquisition and interpretation of data, manuscript writing.
³PhD, Associate Professor, Department of Biological Sciences, Universidade Estadual de Santa Cruz, Ilhéus-BA, Brazil. Interpretation of data and critical revision.
⁴Head and Full Professor, Plastic Surgery Division, UNIFESP, Researcher 1A-CNPq, Director Medicine III-CAPES, Sao Paulo-SP, Brazil. Scientific and intellectual content of the study, interpretation of data and critical revision.

ABSTRACT

Purpose: To evaluate the effects of the adipose-derived stem cells (ADSC) in the viability of random skin flap in rats.

Methods: Thirty five adult male Wistar rats (weight 250–300 g) were used. ADSC were isolated from adult male rats (n=5). ADSC were separated, cultured and then analyzed. A dorsal skin flap measuring 10x4 cm was raised and a plastic barrier was placed between the flap and its bed. After the surgical procedure, the animals were randomized into two groups (n=15 each group), group control and group ADSC. In all groups the procedures were performed immediately after the surgery. The percentage of flap necrosis was measured on the seventh postoperative day.

Results: The ADSC were able to replicate in our culture conditions. We also induced their adipogenic, osteogenic and chondrogenic differentiation, verifying their mesenchymal stem cells potentiality in vitro. The results were statistically significant showing that the ADSC decreased the area of necrosis (p<0.05).

Conclusion: The cells demonstrated adipogenic, osteogenic and chondrogenic differentiation potential in vitro. The administration of adipose-derived stem cells was effective to increase the viability of the random skin flaps in rats.

Key words: Surgical Flaps; Rats; Adult Stem Cells; Stem Cells; Adipose Tissue
Introduction

The stem cells are characterized by their undifferentiated state and their ability to not only generate new stem cells but also specialized cells with different functions. Stem cells can be embryonic or adult. Stem cells derived from adipose tissue are the pluripotent type. In this case these cells can differentiate into osteocytes, chondrocytes, adipocytes, muscle cells, neural and angiogenic lineages.

The beginning of the study of stem cells occurred to the researchers Ernest McCulloch and James Till at the Ontario Cancer Institute in Toronto. Their research reported on the presence of self-renewing cells in bone marrow of mice, and these cells were postulated as regenerative stem cells.

In 2001 stem cells derived from adipose tissue (ADSC) were added to the group of adult stem, showing that they are able to differentiate into mesodermal cells (adipocytes, chondrocytes, osteocytes, and myocytes).

Nowadays it is known that the ADSCs have the ability to form consistent cells as neurons, Oligodendrocytes, Schwann cells and epidermal cell lineage.

The clinical use of this cell type may vary from angiogenesis and neurogenesis stimulation in spinal cord injury to the suppression of the inflammatory response, oxidative stress, and apoptosis in rodent models of ischemia and reperfusion.

The partial necrosis of the skin flaps remains a significant problem in plastic surgery. Recent studies on addition of stem cells from adipose tissue in subcutaneous tissue of rats demonstrate increased vascularity and viability of skin flaps. The aim of this study was to evaluate the effects of adipose-derived stem cells on the viability of random skin flap in rats.

Methods

This project was approved by the Ethics Committee of UNIFESP-CEUAN251501. Animals (250g–300g) were anesthetized with an intraperitoneal injection of 60 mg/kg of ketamine and 5 mg/kg xylazine. The dorsal random skin flap, measuring 10X4 cm, following experimental model proposed by was raised from the deep fascia, including the superficial fascia, panniculus carnosus, subcutaneous tissue, and skin. After flap elevation, a plastic barrier (polyester/polyethylene), with the same dimensions (10X4 cm) was placed between the skin flap and its bed. The flap was then sutured back in place with simple 4-0 nylon sutures. Subsequently, 5X10⁶ ADSC in PBS (0.5 mL) were slowly injected into the caudal vein over 3 min using an insulin syringe in the group experimental. Control group of animals received only 0.5 mL of PBS.

Macroscopic analysis of necrosis percentages

The percentage of skin flap necrosis was measured on the seventh postoperative day, using the paper template method described by. After anesthesia, each flap’s limit between viable skin and necrosis was delineated with a pen. The viable tissue limit has been characterized by soft skin, pink, warm and haired, and necrotic tissue by stiff, dark cool, and hairless skin.

Isolation, culture and expansion of ADSC

ADSC were isolated from adult male Wistar rats (weight 250 – 300 g, n = 5). Rat adipose tissue from inguinal region was enzymatically dissociated for 30 min at 37 °C by 0.1 % (w/v) collagenase type I (Sigma-Aldrich). After centrifugation, the stromal cell pellet was resuspended in Dulbecco’s Modified Eagle’s Medium/Nutrient Mixture F-12 Ham (DMEM/F12) (Sigma-Aldrich) supplemented with 10% Fetal Bovine Serum (FBS) (Cultilab, Campinas-SP, Brazil), 100 U/ml penicillin (Sigma-Aldrich) and 0.1 mg/ml streptomycin (Sigma-Aldrich). The culture was maintained at 37°C in humidified atmosphere of 95% O2 and 5% CO2 and passages with trypsin/EDTA (Gibco) when required. Cells at passage 3 or below were used for experimentation.

Differentiation assays

Differentiation assays was done according to the method described by Gaiba et al 2012. Osteogenic, adipogenic and chondrogenic differentiation were performed to ascertain multipotency of isolated cells. The cells cultured in differentiation media for 21 days. After that, the cultures were stained by a solution of Alizarin, Oil Red O and Toluidine Blue for osteogenic, adipogenic and chondrogenic differentiation, respectively. The fixed and dyed cells were observed using Nikon Ti-U optical microscope and photographed using the NIS-Elements - 3.2 Software (Nikon Instruments INC, New York).

Statistical analysis

The results are expressed as mean ± SD. Comparison between two means was performed by unpaired Student’s t-test. All data were analyzed using GraphPad Prism 3.0 software. Statistically significance was accepted when P<0.05.
Results

Macroscopic analysis of necrosis percentages

The regions of survival and necrosis were clearly demarcated in every flap at 7th day post operation. Figure 1 presents means and distribution of data obtained for percentages of flap viability in the groups. The percentages of viability area (mean ± standard deviation) in the ADSC and control groups were (58.14 ± 4.460)% and (38.86 ± 5.021)% respectively.

Discussion

The use of ADSCs capable of differentiate into mesodermal cells is made since 2001. Their applicability in experimental models is increasing and it is approaching clinical practice.

In plastic surgery this type of cell has also been studied and increasingly used, for example, to increase the success rate of viability in the grafts and small defects in fat grafting. Studies have been done in rats comparing qualitatively peritoneal and inguinal region tissues, however there are no studies that quantitatively compare the number of ADSC in these regions. Thus, interpreting the results we can infer that the number of stem cells in the inguinal region predominates over the peritoneal region (data not shown).

The peritoneal fat, have lower gain of adipose tissue mass compared to lower regions of the body, as the inguinal region, due to a protective mechanism that aims to reduce the metabolic consequences of weight gain. The statistically significant results comparing both collected areas directs the ADSC extraction from the inguinal region, ensuring greater concentration of cells collected in comparison to the peritoneal region, which in turn can be useful in designing future studies aimed at testing the properties of ADSC, as done in this work, which envisaged its closest application to clinical practice.

Regarding the clinical applicability of stem cells, the cutaneous flap is a common and valuable procedure in plastic surgery, such as the repair of retractions of burns and reconstructions after oncologic resections. However, there are factors such as ischemia and necrosis, which may damage its development, justifying the need to investigate possibilities to reduce these risks and increase the viability of the flap.

Studies with models of grafts and flaps using the inguinal region ADSC showed increased viability of the necrotic area, however the route of administration of ADSC was subcutaneously. In the present study, the route of administration was intravenous, finding similar results to those mentioned, which show an increase in flap viability with the use of ADSC.

The statistically significant results regarding the use of ADSC from the inguinal region, decreasing skin flap necrosis, contribute to approximate the experimental use to clinical practice. However, further studies are needed to complement these results, such as the realization of immunohistochemical markers seeking whether there is an increased local vascularization of the flap and the presence of stem cells applied in the flap area.
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Conclusion

The cells demonstrated adipogenic, osteogenic and chondrogenic differentiation potential in vitro. The administration of adipose-derived stem cells was effective to increase the viability of the random skin flaps in rats.

References


Correspondence:
Lydia Masako Ferreira
Disciplina de Cirurgia Plástica-UNIFESP
Rua Napoleão de Barros, 715/4º andar
04042-002 Sao Paulo - SP Brasil
Tel.: (55 11)5576-4118
Fax: (55 11)5571-6579
sandra.dcir@epm.br
silvanagaiba@gmail.com

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