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Qualitative and quantitative differences of adipose-derived stromal cells from superficial and deep subcutaneous lipoaspirates: a matter of fat.

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Abstract

BACKGROUND AIMS:
Subcutaneous fat represents a valuable reservoir of adipose-derived stem cells (ASCs) in the stromal vascular fraction (SVF), widely exploited in regenerative medicine applications, being easily harvested through lipoaspiration. The lack of standardized procedures for autologous fat grafting guided research efforts aimed at identifying possible differences related to the harvesting site, which may affect cell isolation yield, cell growth properties and clinical outcomes. Subcutaneous fat features a complex architecture: the superficial fascia separates superficial adipose tissue (SAT) from deep layer tissue (DAT). We aimed to unravel the differences between SAT and DAT, considering morphological structure, SVF composition, and ASC properties.

METHODS:
SAT and DAT were collected from female donors and comparatively analyzed to evaluate cellular yield and viability, morphology, immunophenotype and molecular profile. ASCs were isolated in primary culture and used for in vitro differentiation assays. SAT and DAT from cadaver donors were also analyzed through histology and immunohistochemistry to assess morphology and cell localization within the hypoderm.

RESULTS:
Liposuctioned SAT contained a higher stromal tissue compound, along with a higher proportion of CD105-positive cells, compared with DAT from the same harvesting site. Also, cells isolated from SAT displayed increased multipotency and stemness features. All differences were mainly evidenced in specimens harvested from the abdominal region. According to our results, SAT features overall increased stem properties.

CONCLUSIONS:
Given that subcutaneous adipose tissue is currently exploited as the gold standard source for high-yield isolation of adult stem cells, these results may provide precious hints toward the definition of standardized protocols for microharvesting.

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KEYWORDS:

adipose-derived stem cells; regenerative medicine; stemness; subcutaneous adipose tissue

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Harvesting technique affects adipose-derived stem cell yield.

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Abstract

BACKGROUND:
The success of an autologous fat graft depends in part on its total stromal vascular fraction (SVF) and adipose-derived stem cells(ASCs). However, variations in the yields of ASCs and SVF cells as a result of different harvesting techniques and donor sites are poorly understood.

OBJECTIVE:
To investigate the effects of adipose tissue harvesting technique and donor site on the yield of ASCs and SVF cells.

METHODS:

Subcutaneous fat tissues from the abdomen, flank, or axilla were harvested from patients of various ages by mechanical liposuction, direct surgical excision, or Coleman's technique with or without centrifugation. Cells were isolated and then analyzed with flow cytometry to determine the yields of total SVF cells and ASCs (CD11b-, CD45-, CD34+, CD90+, D7-FIB+). Differences in ASC and total SVF yields were assessed with one-way analysis of variance. Differentiation experiments were performed to confirm the multilineage potential of cultured SVF cells.

RESULTS:
Compared with Coleman's technique without centrifugation, direct excision yielded significantly more ASCs ($P < .001$) and total SVF cells($P = .007$); liposuction yielded significantly fewer ASCs ($P < .001$) and total SVF cells ($P < .05$); and Coleman's technique with centrifugation yielded significantly more total SVF cells ($P < .005$), but not ASCs. The total number of SVF cells in fat harvested from the abdomen was significantly larger than the number in fat harvested from the flank or axilla ($P < .05$). Cultured SVF cells differentiated to adipocytes, osteocytes, and chondrocytes.

CONCLUSIONS:
Adipose tissue harvested from the abdomen through direct excision or Coleman's technique with centrifugation was found to yield the most SVF cells and ASCs.

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Long-term culture optimization of human omentum fat-derived mesenchymal stem cells.

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Abstract

Recent scientific explorations in search of novel sources for autologous transplantation transpired an alternative source of MSCs (mesenchymalstem cells) derived from omentum fat. The scarcity of experimental evidences probing into the biosafety concerns of omentum fat-derived MSC under prolonged culture conditions limits its applicability as an efficient tool in regenerative medicine. This study, thus, aims to optimize human omentum fat-derived MSC in four different media [DMEM (Dulbecco's modified Eagle's medium) LG (low glucose), DMEM KO (knock out), α-MEM (α-minimal essential media) and DMEM F12] in the facets of phenotypic characterization, growth kinetics, differentiation and karyotyping under prolonged culture. The cells exhibited a similarity in expression profile for the majority of markers with evidential variations in certain markers. The relevance of omentum fat-derived MSCs became evident from its triumphant differentiation potential and karyotypic stability substantiated even at later passage. The results obtained from growth curve and PDT (population doubling time) lead to optimization of appropriate media for omentum fat-derived stem cell research, thereby bringing omentum fat into the forefront of regenerative medicine.

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Zhonghua Shao Shang Za Zhi. 2014 Dec;30(6):512-7.

[Effects of rat allogeneic adipose-derived stem cells on the early neovascularization of autologous fat transplantation].

[Article in Chinese]
Tian T[1], Jia C[2], Liu Y[1], Liu Z[1], Hu G[1], Wang R[1], Chang C[1].
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Abstract

OBJECTIVE:
To investigate the effects of allogeneic adipose-derived stem cells (ADSCs) of rat on the early neovascularization of autologous fat transplantation.

METHODS:

(1) Experiment 1. Adipose tissue was collected from both inguinal regions of two SD rats to isolate, culture, and purify ADSCs through collagen enzyme digestion, density gradient centrifugation, and adherence method. The fourth passage of cells were collected for morphologic observation, detection of expressions of surface markers CD34, CD49d, CD106, and CD45 of ADSCs with flow cytometer, identification of adipogenic and osteogenic differentiation, and determination of the cell proliferation ability with thiazolyl blue method. (2) Experiment 2. Another 30 SD rats were divided into allogeneic adipose granule (AG) group (A, n = 6), autologous AG group (B, n = 8), autologous ADSCs+autologous AG group (C, n = 8), and allogeneic ADSCs+autologous AG group (D, n = 8) according to the random number table. The fourth passage of ADSCs were obtained from adipose tissue from one side of inguinal region of SD rats in group C. Adipose tissue obtained from one side of inguinal region of SD rats of the other 3 groups was abandoned. The AG was prepared from another side of inguinal region of SD rats in the 4 groups. The mixture of 0.6 g AG from one rat and 1 mL DMEM/F12 nutrient solution was injected subcutaneously into the back of another rat in group A, and so on. Autologous AG was injected into its own body of the rats in group B. The mixture of 1 mL autologous ADSCs mixture which contains 3.0×10^6 cells per mililitre autologous ADSCs combined with autologous AG was injected into the rats in group C. The mixture of 1 mL allogeneic ADSCs mixture which contains 3.0×10^6 cells per mililitre ADSCs extractived from the former 2 rats in experiment 1 combined with autologous AG was injected into the rats in group D. At 7 days post transplantation, fat transplants were harvested for gross observation, measurement of wet weight, pathological observation, and assessment of cells with positive expression of CD31 with immunohistochemical method. Data were processed with one-way analysis of variance and SNK test.

RESULTS:

(1) The fourth passage of cells proliferated well showing fusiform shape similar to fibroblasts. These cells showed positive expression of CD34 and CD49d and weak positive expression of CD106 and CD45. They were able to differentiate into adipocytes and osteoblasts. These cells were identified as ADSCs. The fourth passage of cells grew faster than that of the tenth passage. (2) At 7 days post transplantation, no liquifying necrosis or infection was observed in the fat transplants of the rats in the 4 groups. Wet weight of the fat transplants in groups A and B was respectively (0.25 ± 0.04) and (0.26 ± 0.03) g, which were less than those of groups C and D [(0.36 ± 0.03) and (0.35 ± 0.04) g, with P values below 0.05]. HE staining showed that there were less fat cells and more fibroblasts in the transplants of group A, visible fibrous tissue around uneven shape of fat cells in the transplants of group B, and almost identical size and shape of fat cells and unobvious fibrous tissues were found in the transplants of groups C and D. The cells with positive expression of CD31 were distributed in fibrous tissues in larger number but less around fat cells in the transplants of group A, while more of these cells were observed surrounding fat cells in the transplants of group B. There were more cells with positive expression of CD31 distributed surrounding fat cells in the transplants of groups C and D than that in group B. The cells with positive expression of CD31 observed under 400 times field were more in number in groups C (20.5 ± 1.1) and D (22.1 ± 1.0) than in groups A (8.0 ± 3.6) and B (10.9 ± 1.7), with P values below 0.05.

CONCLUSIONS:

Allogeneic ADSCs combined with autologous AG can significantly improve the early vascularization of fat transplantation as well as autologous ADSCs combined with autologous AG.

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