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Case Report Open Access

Yielding and Growing of Adipose Stem Cell Harvested from the Superficial and Deep Abdominal Subcutaneous Fat: A Case

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Abstract

A 60 year old woman was enrolled in our study. She was 151 cm tall, weighed 73 kg and had high body mass index (33.19 kg/m^2). Adipose tissue specimens were taken from her abdomen by surgical excision. ASCs were isolated in primary culture and cell counts were performed by using an automated cell counter (LUNA™). And 5 × 10^3 ASCs were seeded into DMEM tissue culture flasks and were cultured for 16 days (n=3). Lastly, self-renewal capacity was demonstrated by colony-forming unit fibroblast (CFUF) assays (n=3).

Keywords: Adipose stem cell; Subcutaneous fat

Case Report

Subcutaneous fat is a valuable source of adipose-derived stem cells (ASCs). There are two anatomically distinct compartments of subcutaneous fat tissue in human: superficial adipose tissue (SAT) and deep adipose tissue (DAT). Abdominal subcutaneous fat is also separated by scarpa's fascia into two layers, which are SAT and DAT. The difference between SAT and DAT had been studied in anatomy [1,2], structure and function [3], insulin resistance [4], molecular and morphologic character [5] and metabolic markers [6]. Taranto et al. recently demonstrated that SAT contained a higher stromal tissue compound and features increased stem properties [7].

We have observed yielding and growing of ASCs derived from SAT and DAT. The entire protocol was approved by the institutional review board (No. 12-146) at the Dongsan Presbyterian Hospital (Daegu, Republic of Korea). A 60 year old woman was enrolled in our study. She was 151 cm tall, weighed 73 kg and had high body mass index (33.19 kg/m^2) and was admitted for breast reconstruction. Adipose tissue specimens were harvested from zone IV of transverse rectus abdominis musculocutaneous (TRAM) flap and were separated into SAT and DAT by surgeon (author) at operating room. The specimens were transferred to laboratory immediately for preparation.

The purified fat was minced and washed with PBS, and digested with type I collagenase for 40 min. Equal amount of DMEM (10% FBS) was added to stop enzyme action. The digested tissue was filtered through a 200 μm strainer. After two centrifugations ASCs were isolated in primary culture and cell counts were performed three times for every sample by using an automated cell counter (LUNA™, Logos biosystems, Anyang, South Korea). And 5 × 10^3 ASCs were seeded into DMEM tissue culture flasks and were cultured for 16 days. Lastly, self-renewal capacity was demonstrated by colony-forming unit fibroblast (CFU-F) assays.

For growth curve analysis, cell counts were compared by *t*-test for individual comparisons (Figure 1).

Total cell count of ASCs derived from DAT was higher than SAT group but live cell count of ASCs derived from DAT was lower than SAT group (Table 1). Cell growth rate of DAT group was higher than SAT group and last cell count of DAT group was higher than SAT group (Figure 1). CFU-F assays revealed that SAT group formed fewer and smaller colonies but DAT group formed multiple and larger colonies. And optical density of DAT group was higher than SAT group. This means that self-renewal capacity of ASCs derived from DAT was better than SAT group (Figures 2 and 3). In summary, SAT contained a higher amount of ASCs but the growth rate and self-renewal capacity were lower than DAT.

Type	Total fat weight (g)	Total cell yield/g	Live cell yield/g
DAT	117.0	1.8 × 10^4 ± 9.8 × 10^3 cells	6.3 × 10^3 ± 8.8 × 10^2 cells
SAT	180.3	1.1 × 10^4 ± 1.9 × 10^3 cells	7.6 ×10^3 ± 1.4 × 10^3 cells

Table 1: Yield of adipose derived stem cell; total cell count of ASCs derived from DAT was higher than SAT group but live cell count of ASCs derived from DAT was lower than SAT group.

Harvesting of DAT from SAT is not an easy procedure. A scarpa's fascia can be clearly demonstrated under CT and MRI but in clinical field it is almost impossible to harvest only deep abdominal fat using a suction cannula during liposuction. Even in the excised full layer of abdominal skin and subcutaneous fat the scarpa's fascia is unclear medially. Especially in lower BMI patients who have thin DAT layer, harvesting is even more difficult.

As far as we are aware, this is the first study of comparing growth rate of SAT and DAT. Our case study showed that DAT has better growth and self-renewal capacity than SAT. This result was different from Taranto et al. study [7]. Although our study has a limit as a case study, these results may provide a clue to further study about SAT and DAT.

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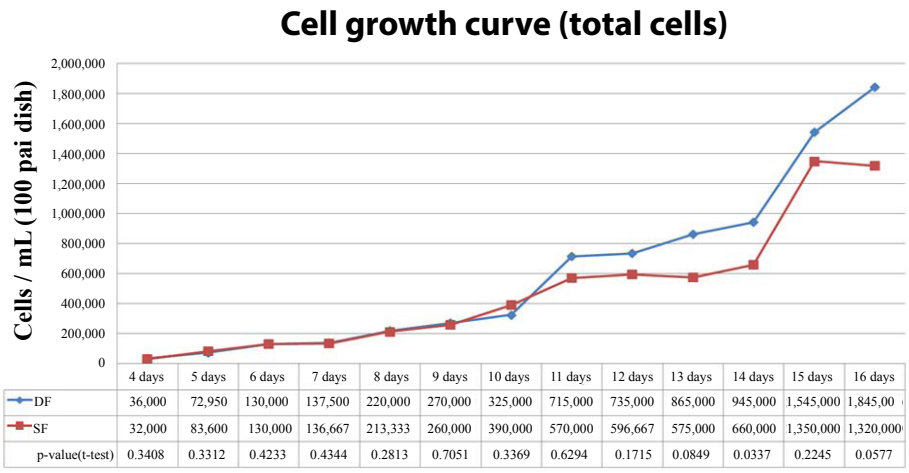

	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days	15 days	16 days
DF	36,000	72,950	130,000	137,500	220,000	270,000	325,000	715,000	735,000	865,000	945,000	1,545,000	1,845,00
SF	32,000	83,600	130,000	136,667	213,333	260,000	390,000	570,000	596,667	575,000	660,000	1,350,000	1,320,000
p-value(t-test)	0.3408	0.3312	0.4233	0.4344	0.2813	0.7051	0.3369	0.6294	0.1715	0.0849	0.0337	0.2245	0.0577

Figure 1: Cell growth curve; cell growth rate was similar until 10 days of culture, but DAT (DF) group was higher than SAT (SF) group after 11 days of culture and last cell count of DAT group was higher than SAT group.

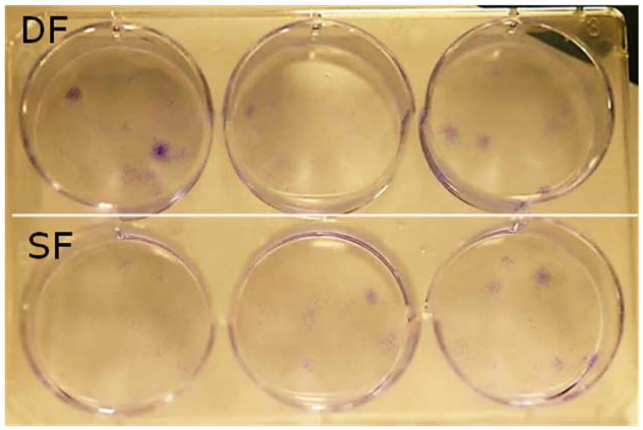

Figure 2: Colony-forming unit fibroblast (CFU-F) assays; cell colonies were stained with 1% crystal-violet in methanol at 15th day of culture. SAT (SF) groups (lower three culture dishes) established fewer and smaller colonies but DAT (DF) groups (upper three culture dishes) established multiple and lager colonies.

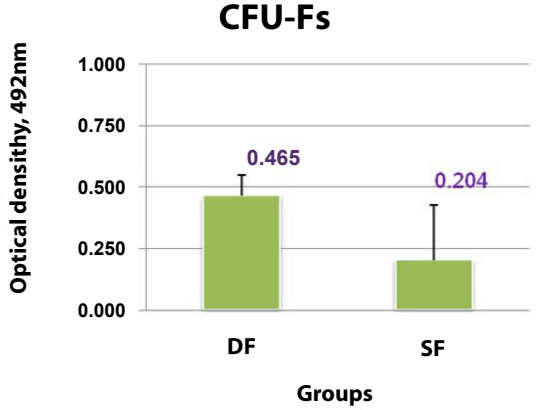

Figure 3: Optical density of Colony-forming unit fibroblast (CFU-F) under illumination at a wavelength of 492 nm; optical density of DAT group (mean, 0.465) was higher than SAT (SF) group (mean, 0.204). This means that self-renewal capacity of ASCs derived from DAT (DF) was relatively two times higher than ASCs derived from SAT.

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