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Counting Method of Live Fat Cells Used in Lipoinjection Procedures

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Abstract. It has been possible to determine the number of live fat cells available in samples of aspirated fat which are going to be used in lipoinjection procedures. It has been possible to develop a technical procedure that quantifies the number of live fat cells in 1 mm of injection. Without taking into account different methods for fat injection, there is a 95% correct conclusion that it is immaterial whether the syringe or a cannula with a pump is used. However, the higher the amount of blood found in the samples being used for injection, the less amount of live fat cells found.

Key words: Lipoinjection procedures—Determination of survivable live fat cells.

Introduction

The introduction of lipoplasty in the plastic surgery armammentarium brought conditions to eliminate fat deposits leaving inconspicuous scars. Soon the experience shows the importance of how much fat can be removed and how much is to be left, based on a narrow limited equilibrium line, where the skin turgor, the fats previous volume, and the body region, interfere in the quality of the results. Fat suction beyond these limits brought secondary effects on the skin quality combined with waves, dents, and laxity. The use of fat injection to solve these problems reactivated the concepts of adipous tissue autografts. Concomitantly, silicone and collagen injection also started to be used in an attempt to solve these problems. Until now the results already published with the material injected (fat, silicone, collagen, etc.) have been controversial. Great variations in the reabsorption, integration, plus the presence of systemic and collateral effects, still show an unclear understanding about the problem.

The fat injection is still in use, while the silicone and collagen injections are losing their credibility among patients and specialists. Today, the fat harvesting is performed with syringe or cannula. In both situations, there is a negative pressure inside the syringe or in the pump connected to the cannula to make the vacuum for the fat removal. Before being injected, this fat is prepared by different methods, the most frequent of which are wash with saline solution, decantation, centrifugation, and drugs to be mixed with the fat.

Until now, there is a clear evidence of lack of knowledge about the number of live fat cells injected and the number that effectively will integrate. The present article has the scope to study and improve how many fat cells are effectively injected with the use of the cannula and syringe, and the higher ratio of live cells that each one can offer.

Revision of the Literature

The first report of fat autograft reports the use of the omentum graft inserted between the liver and the diaphragma [1]. Fat tissue fragments graft were used to solve small skin dents [1], conduct of fat tissue harvesting, fat injections for problems of face hemiatrophy deformity, and for face wrinkles were also published [1–5,9]. Studies of the correct use of negative pressure to better preserve the fat tissue harvesting, the cryoanesthesia in the donnor site, and the use of fat homograft injections were evaluated and published [6,7,11,13]. Care with fat tissue manipulation, insulin efficacy, average of fat reabsorption, fat reinjections, late complications after fat injections, and fat wash with saline solution were also published [3,4,8,10–12,14]. Research in rabbits that compared fat block grafts and fat injections report high absorption levels [15,16].

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Materials and Methods

Forty samples of human fat tissue were obtained from 20 patients admitted to in-hospital aesthetic or reconstructive surgery. Age, sex, body weight, and body region were not taken into account. In all cases, no local anesthesia was used. In 20 samples, Liposuction was performed with a 10-cc syringe and cannula of 4 mm external diameter in 20 and 10 samples, respectively. The syringe's internal negative pressure was 510 mm/Hg measured by an aneroid vacuummeter. In an additional 20 samples, the fat was obtained with a 4-mm external diameter cannula adapted to a pump with similar negative pressure.

All the suctioned fat tissue (by syringe and cannula) were brought to the laboratory inside of a 10-cc regular syringe. In all syringes the fat tissue remained separated on an upper layer from a nondense stratum of blood and oil.

Tissue was stored in a refrigerator for a minimum period of 1 day and a maximum of 6 days. After this time period, each sample received the same treatment: [1] The fat was transferred to a siliconized separator funnel and washed with 0.85% of saline solution. Continuous and delicate circular movements were carried out in the funnel to remove all other nonfat materials [2]. From the fat final volume (twice weighted to reconfirm its effective weight), 0.5 g were separated and transferred to a 10-ml beaker where 0.5 ml of 0.85% of saline solution and (10:5-v/p) of collagenase was added [3].

This solution was placed for 2 h in a stove at 37°C. The positioning of the beaker was occasionally readjusted by hand [4]. After its removal from the stove, the solution was mixed with a glass stick, and the suspended material was removed with delicate forceps from the solution [5]. The solution was centrifuged for 1 min at 600 rpm. The supernatant was aspirated with syringe and the remaining floating liquid was transferred to a 0.5 ml volummetric balloon, and the volume was completed with 0.85% of saline solution [6]. A red blood cell counter pipette was filled to the 0.5 mark by capillarity with the fluid from the flask. Sudan Black is then drawn to the 21 mark of the same pipette [7]. At 30 min later, the solution was transferred to a RBC (red blood cell counter) to count the total stained and nonruptured fat cells. The counting was conducted in a Neubauer chamber in the similar way used for the white blood cells of a leucogram.

An equation was used in order to calculate the number of cells in the chamber: number of cells versus height in the chamber versus dilution where height equal to 10 and dilution equal to 200. Mann–Whitney nonparametric ''U'' test was used for statistical analysis of data. This maneuver was repeated in all 40 samples processes.

Results

All 40 fat samples were submitted to the same experimental method. In all samples, floating oil and blood with different respective volumes were observed. The results were registered according to the manner of fat removal and the chronology of treatment (Table 1). The level of statistical comparison between two types of samples was ''U'' = 191.5, where N1 is of syringe samples, and N2 liposuction by cannula samples. ''U'' critical 127 for N1 = 120 and for N2 = 20 alfa level of 0.05.

Table 1. Counting method of line fat cells

Sample	Cannula/syringe	Number fat cells/MM3
1	Cannula	19.000
2	Syringe	13.000
3	Cannula	9.000
4	Syringe	6.000
5	Cannula	5.000
6	Syringe	18.000
7	Cannula	18.000
8	Syringe	13.000
9	Cannula	16.000
10	Syringe	9.000
11	Cannula	13.000
12	Syringe	36.000
13	Syringe	6.000
14	Cannula	12.000
15	Cannula	9.000
16	Syringe	18.000
17	Cannula	23.000
18	Syringe	42.000
19	Syringe	33.000
20	Syringe	20.000
21	Cannula	30.000
22	Cannula	12.000
23	Syringe	13.000
24	Syringe	16.000
25	Cannula	11.000
26	Cannula	13.000
27	Syringe	16.000
28	Syringe	9.000
29	Cannula	19.000
30	Cannula	10.000
31	Syringe	12.000
32	Syringe	18.000
33	Cannula	7.000
34	Cannula	19.000
35	Cannula	14.000
36	Syringe	12.000
37	Cannula	16.000
38	Cannula	10.000
39	Syringe	7.000
40	Syringe	10.000

Statistically, the samples were considered equal in 95% of probability. The live fat cells obtained with the cannula was 14.000/mm^3 with the pattern deviation of 5.970 cells approximately. The live fat cells obtained with syringe was 15.700/mm^3 with a pattern deviation of 10,000 cells approximately.

Comments

The technique objective is the evaluation of the amount of live fat cells obtained from the most usual methods of

fat collection to be harvested and injected as free graft. Sex, race, nutritional conditions, and body weight were not taken into account. The fast samples were obtained from selected patients without any pathology that could bring modifications in the subcutaneous tissue normal structures.

The fat tissue was always obtained form the subcutaneous deep level (below the superficialis fascia). The anesthetic methods used were under general, peridural, or regional block anesthetic. No local anesthetic infiltration was used to avoid damage to the cells vitality or its percentage amount in the samples. The vacuum inside of the 10-, 20-, and 50-ml syringe were tested with an aneroid vacuummeter similar to the current pump used for liposuction. After 10 repeated tests in each type of syringe, the final rates were 510 mm/Hg for the 10-ml syringe; 630 mm/Hg for the 20-ml syringe and 720-mm/Hg for the 50-ml syringe. The pump negative pressure used for the cannula was always around 500 mm/Hg. The pump and 10-ml syringe with similar negative pressure (around 500 mm/Hg and with a similar cannula 4 mm diameter outside and 32 mm diameter inside were used to collect the fat in all 40 samples (20 with pump and 20 with syringe). The thin cannula diameter adapted in the syringe and in the pump, was specifically selected due to its easy condition of work in every region of the body.

A revision in all the 40 samples, shows that the blood amount was different macroscopically. Microscopically the fat cells amount decreased with an increase in blood amount. In all fat samples collected by pump, there was a long silicone tube between the pump and the cannula. Liposuction harvests the fat into the silicone tube immediately connected to the cannula until the desired volume (around 15 ml of fat) is reached. After it, the silicone tube was angulated, the pump turned off, the silicone tube disconnected from both the pump and the cannula. The proximal segment of the silicone tube was then turned downward and its ''mouth'' inserted into a 10-ml syringe. The aspirated fat was dropped into the syringe until reaching 10 ml. The fat samples numbered from 1 to 40 were sent to the laboratory without identification if it were obtained with syringe or pump. Since it was not possible for the laboratory personnel to process the material immediately upon receipt, it was stored in a refrigerator at 2°C during a period of 1–6 days to avoid cellular destruction. All samples presented an oil floating material that was analyzed by gaseous chromatography to examine its nature. After these procedures, the substances identified were lauric acid, stearic acid, palmitic acid, linoleic acid, palmioleic acid, miristic acid, oleic acid, and araquidic acid. The palmitic acid presented the highest volume among them.

Robdell's [12], technique proved to be the best one to isolate the fat cells. All the fat samples were washed with 0.85% of saline solution for the excess of blood removal. Some of them received up to 3 l of this saline solution. This procedure was taken into account only to remove the blood excesses mixed with the fat cells, and not as Chajchir [3] says, which is, ''that the saline solution changes the cells morphology.''

The silicone in the separator funnel was used only for lubrication and in a minimum amount to facilitate the technical performance.

When the collagenase was added and the material was inserted in the stove for incubation, an electromagnetic agitator was used and then abandoned based on a high cellular destruction. Gently manual movements substituted this procedure.

The score of the fat cells after isolated was similar to those used in the current hemogram counting method. The fat cells coloration was obtained with Sudan Black from a saturated solution previously filtered (filtrum paper) to remove its pigment excesses.

At the score stage, the fat cells were separated one from another by its round shape, and with a great volummetric difference between them, reaching up to 10 times. Mann–Whitney ''U'' nonparametric method was selected by its efficiency when the ''*t*'' Student method cannot be used. In spite of the great number of live fat cell variation in each sample, it was possible to estimate their number in 1 ml and then to 10 ml of collected fat. These results should reflect in the routine of the fat graft injection, based on the amount of different material that is also injected with the live fat cells that were not analyzed (oil, destructed cells, proteins, etc.). The samples obtained with syringe presented 15,700/mm^3 with a pattern deviation of 10.000 cells, while the samples obtained with pump presented an average of 14,000 cells/mm^3 with a pattern deviation around 5,950 cells. These results were obtained after elimination of all the floating oil, blood, protein, destructed cells, and other nonidentified substances. No specific evaluation as taken into account relative to different methods for fat injection with the use of previous centrifugation or chemical use of substance-like insuline.

The method used may bring a better knowledge of what quantitatively live fat cells are effectively injected. The great discrepancy in the fat injection results shows that an ideal method to perform it still does not exist.

Fredericks [6], reports that probably the fat injection may not be the definitive solution in the deformities treatment as they have being used. It is possible that the quantitatively live cell injection studies could be more effective and less empiric for the results evaluation.

Conclusions

It is possible to determine the number of life fat cells in all the samples collected. It was also possible to develop a technical procedure to quantify the number of live fat cells in 1 mm. Without taking into account different methods for fat injection, there is a 95% correct conclusion that it is immaterial whether the syringe or a cannula with a pump is used. The higher amount of blood found in the samples, the less amount of live fat cells can be found. The average of integrated live fat cells presented in a sample collected with syringe were of 15,700/mm^3. The average of integrated live fat cells present in the samples collected with a cannula and pump were of 14,000/mm^3.

Summary

The present subject demonstrates that it is possible to quantify the number of live fat cells in 1 ml, obtained with liposuction with cannulae and syringe. Statistically, there is not a significant difference in the selection of the methods and the number of live fat cells.

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