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Comparison of Viability of the Preserved Autologous Aspirated Fat Tissue Transfer in Refrigerated and Frozen Specimens

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Abstract

Background: Fat graft surgery is one of the most effective procedures in plastic surgery, and since some patients request multiple surgeries and these cases sometimes take hours, it endangers the viability of the fat graft. In this study, we intend to evaluate the viability of adipose tissue aspirated with a syringe at refrigerator (4°C) and freezer (–20 °C) temperatures.

Methods: This was a cross-sectional study. After receiving the ethics committee's approval (IR.MUMS.MEDICAL.REC.1401.423), 17 volunteers entered the study. The harvested fat tissue sample was divided into 3 parts, and each of them was transferred to 3 separate sterile tubes. The first tube was sent to the laboratory for preliminary examination of fresh fat, and the second tube was transferred to a 4°C refrigerator for 72 hours. The sample from the third tube was first passed through a strainer and after drying, it was transferred to a –20°C freezer for 72 hours. After treatment with trypsin, we placed the sample inside the centrifuge using the Coleman method. Finally, 3 layers were formed, and the white middle layer was extracted as a fat cell suspension. Tissue samples were stained with trypan blue, and the percentage of viable cells was calculated using an optical microscope.

Results: There was a significant difference between the mean number and percentage of viable cells in all 3 groups. Samples in the 4°C refrigerator had significantly more cellular viability than those in the –20°C freezer (mean difference, 72.842%; $P < 0.001$).

Conclusion: Our findings showed that after 72 hours at 4°C, adipose tissue has significantly higher survival than at –20°C (98.93% vs 75.31%). Since the survival of fat cells is one of the direct determinants of fat retention, it can affect the results after surgery. The present study recommends fresh adipose tissue for immediate transplantation unless there is an urgent need for cold storage.

Keywords: Temperature of Storage, Adipocyte Survival, Cell Viability

Conflicts of Interest: None declared
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Introduction

Because of its availability, simplicity in harvesting, and natural appearance, autologous fat makes a significant soft tissue filler (1). Autogenous fat grafting is a straightforward method to enhance soft tissue in cosmetic and reconstructive plastic surgery. It has numerous donor sites and does not cause immune or foreign body responses. As a result, it has a wide range of applications in plastic and reconstructive surgery, and its clinical use is growing (2).

Various soft tissue defects can be corrected using autologous fat injection. Aesthetic improvement of facial anomalies of congenital and acquired origin, such as Romberg's disease, facial hemi-atrophy, depressed scars, wrinkles, eyelids, depressions, pitting acne, and posttraumatic defects, have been described by this procedure. It has also been described that fat injections can be used to correct irregularities caused by suction-assistant liposuction. Fat tissue becomes suspension fat globules under a negative pressure of 1 atm (3).

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↑What is "already known" in this topic:
Various factors, including centrifugation and washing, can affect the viability of fat tissue cells.

→What this article adds:
Keeping adipose tissue cells at 4°C can increase survival compared with –20°C.

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Although some are damaged or die due to trauma caused by the suction procedure, these cells can be injected subcutaneously as an autologous-free graft and survive at least partially. Injections of fat have low morbidity and can be performed on an outpatient basis without surgical incisions or scarring. A high absorption rate of the injected fat, reaching up to 70% of its volume, is the main obstacle to long-term soft tissue filling. As a result of this high absorption rate, overcorrection and reinjection procedures are necessary (3).

Some patients request multiple surgeries at the same time (for example, liposuction of the abdomen and simultaneous mammoplasty with fat graft in other areas of the body) and in different positions, and these procedures sometimes require hours (sometimes over 6 to 8 hours), thus, it puts the viability of the fat graft at risk. Also, some patients under the influence of social media expect that the extra fat harvested from their body can be stored in a freezer at –20°C and used again in future procedures. The survival rate of the adipocytes is essential when frozen or cryopreserved fat is used after storage because they have a considerable influence on the engraftment of adipose tissue after reinjection (4-11).

In the case of some transplanted organs (for example, heart, lung, and kidney), based on the literature, the organ is kept at a temperature of 4°C for preparation before transplantation (3, 12-16).

It is increasingly more crucial to think about whether cryopreserved adipocytes are a viable substrate for soft tissue augmentation and whether preservation approaches will result in the best surgical outcomes because autologous fat transfer is becoming more and more popular (17). Therefore, due to a lack of data on the viability of fat tissue in different temperatures in previous studies, this study aimed to compare the viability of autologous fat tissue in refrigerated and frozen specimens.

Methods

Study Design

This was a cross-sectional analytical study. After receiving the approval of the ethics committee of Mashhad University of Medical Sciences, 17 middle-aged female (35-45 years) participants were included in the study after receiving written consent and based on purposive sampling. The inclusion criteria for the study included not having specific medical conditions and a body mass index (BMI) of 25 to 30 kg/m^2. The patients were admitted to the plastic surgery department of Ghaem Hospital, affiliated with the Faculty of Medicine of Mashhad University of Medical Sciences. After anesthesia, in the supine position, the abdomen was prepared and draped. Then, 6 cc of Tumescent solution (Hunstad's Formula for Tumescent solution; 1000 cc of ringer lactate + 50 cc of 1% lidocaine + 1 cc of epinephrine 1:1000) was injected into the hypogastric region. After giving 10 minutes for the Tumescent solution to take effect, using a 20-cc syringe connected to a 2 mm cannula, 6 ccs of adipose tissue aspired with gentle pressure.

The resulting sample was divided into 3 parts, and each of them was transferred to 3 sterile tubes (3 cc syringe connected to Lorlac) with a volume of 2 cc. The first tube was immediately sent to the laboratory for a preliminary examination of fresh fat in the first hour. The second tube was transferred to a 4°C refrigerator, and the sample from the third tube was first passed through a strainer, and after drying, it was transferred to a –20°C freezer.

The second tube was kept in a physiological serum in a vertical position (under gravity) for 72 hours in a normal refrigerator at 4°C. The third tube was stored in a freezer at –20 °C (dry-frozen) for 72 hours.

Both the 4°C refrigerator and the –20°C freezer were located in the cytogenetic laboratory of the pathology department and were equipped with an alarm system against power failure.

Pathological Investigation Method

First, 1 cc of aspirated adipose tissue was treated with 1 cc of trypsin solution (Trypsin EDTA 1X 0.25%) and kept in a shaker incubator (ES-20/60/Biosan; Riga, Latvia) at 37°C for 10 minutes.

Next, we put the sample in a centrifuge (iCEN-24; Allsheng) for 3 minutes at a speed of 3000 rpm using the Kalman method. After centrifugation, 3 layers can be seen inside the tube. The connective tissue and trypsin remains are thrown out from the tube by separating the Lorlac. The yellow upper layer is the same oil thrown out by overflowing the tube, and the rest is absorbed by placing filter paper inside the tube. The white middle layer is extracted as a fat cell suspension.

Then, using a sampler, 50 microliters of fat cell suspension was added to 50 microliters of 0.4% Trypan blue solution to reach a 1 to 2 dilutions, and the suspension was prepared using a pipette. The mixture of dye and cells was poured by placing the tip of the pipette in the slot of the hemocytometer slide, and the cover glass was placed on the Neubauer slide so that it was spread entirely. Finally, we started counting cells under a light microscope with a magnification of 100. Note that it is better to read the slide within 3 minutes because trypan blue can damage the cell if it is in contact with the cell for a long time.

Statistical Analysis

The data obtained from the study were entered into the statistical software SPSS Version 20. The Kolmogorov-Smirnov test was used to check the normality of the data distribution. Descriptive statistics—Mean ± SD or frequency (%)—were used to describe the data. Due to the normality of data distribution, analyses were performed using parametric tests (1-way analysis of variance). $P < 0.05$ was considered statistically significant in this study. Multiple comparisons were checked using the Tucky post-hoc test.

Results

A total of 17 patients were included in the study. The patient's age ranged between 35 and 45 years, with a mean of 40.17 years ± 3.12. Patients had a BMI of 25 and 30 kg/m^2, with a mean of 28.05 kg/m^2 ± 1.74.

Table 1 demonstrates the total and viable cells and the percentage of live cells in all 3 specimens. The values for

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Table 1 . Comparison of Viability Between Groups[1]

Variable	Fresh	4°C	-20°C	*P* Value*	*P* Value**	*P* Value***	*P* Value****
Total number of cells	1069.11 ± 278.10	1052.17 ± 268.21	892.29 ± 268.96	0.123	0.982	0.151	0.210
Number of live cells	1057.41 ± 271.83	789.47 ± 194.86	23.05 ± 17.24	< 0.001	0.001	< 0.001	< 0.001
Percentage of viability	98.93 ± 0.66	75.31 ± 4.55	2.47 ± 1.42	< 0.001	< 0.001	< 0.001	< 0.001

[1] * Between groups, ** between Fresh and 4°C, *** Fresh and -20°C, ****4°C and -20°C

viability were higher in specimens stored at 4°C than in specimens stored at –20°C. There was a significant difference between the mean number of viable cells and the viability percentage in all 3 groups.

Discussion

Fat grafting has become very popular as a soft tissue filler. For example, autogenous fat is routinely used in liposuction (18, 19). However, some problems, such as fat tissue analysis, estimated at 20% to 90%, can affect fat grafting (20, 21). The higher the number of living fat tissue cells, the higher the chance of graft survival. Therefore, various harvesting methods have been proposed to solve the problem, preparing and transferring it (22). Thus, the present study compared the viability of transplanting autologous aspirated fat tissue preserved in refrigerated and frozen specimens.

Based on the results of our study, fresh fat tissue is suitable for grafting, and in the case of storage, tissues stored at 4°C are significantly more viable than tissues stored at –20°C (Figure 1). Therefore, to achieve better results, 4°C storage is preferred over –20°C.

The use of autologous fat grafting to repair cosmetic and reconstructive surgery has dramatically increased. Fat grafting is typically considered safe, with high patient satisfaction (23).

In a 2007 study by Erdim et al, adipose tissue samples stored at –20°C for 2 weeks had significantly lower cellulose viability than fresh samples, while the viability of fat cells held at 4°C was not significant compared with new fat (5). In Another study in the same year by Matsumoto Daisuke et al, the adipose tissue obtained from 10 patients was kept at room temperature, and after 4 hours, there was significant damage to adipocytes, while at a temperature of 4°C for 3 days, there was no significant damage (7). Our investigation did, however, reveal a substantial variation in the viability of fresh and refrigerated fat. Son Daegu's study in 2010 demonstrated a rapid and significant decrease in the cellular viability of adipocytes after 24 hours of being stored at –15°C (9). In a 2020 study by Kim Yeon Dong et al, 3 samples of adipose tissue obtained from a patient were stored at –20°C, and after 1 week, no viable tissue was found, while 3 other samples from the same patient were held at 4°C and there was significant viability after 1 week (10).

In the present study, there was a remarkable difference in the viability of fat cells between refrigerated and frozen specimens, as refrigerated specimens were more viable, with a mean difference of 72.842%. Interestingly, studies have reported that in samples stored at –16°C, the inflammatory and necrotic reactions are significantly higher than those stored at +1°C (time-dependently) (24). This issue can be considered partially due to the damage of fat cells during freezing or thawing processes (25). However, it is possible that improvements in freezing methods can increase cell viability. In general, storing fat cells at +4°C is more accessible and compared with freezing, the survival rate of cells is higher.

Conclusion

The present study concluded that autologous fat tissue has a significantly higher cellular viability at 4°C than –20 °C after 72 hours of storage. This study also recommends fresh fat tissue for grafting, as it has considerably more

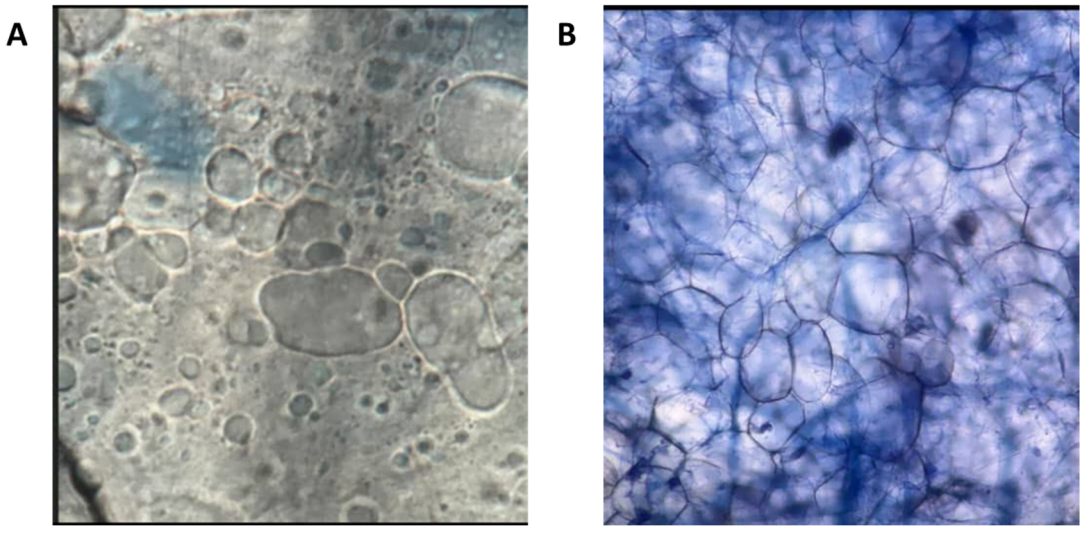

Figure 1. A: Aspirated fat cells were stored at +4°C (after 72 h). B: Aspirated fat cells were stored at -20°C (after 72 h). In trypan blue staining, dead cells are colored (blue).

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viability and better outcomes than cold storage.

Limitations and Recommendations

Based on the literature, the viability of fat tissue varies depending on different methods of harvesting, tissue preparation, freezing, and the number of samples. Thus, to achieve the optimal temperature, it is recommended to evaluate the viability of fat tissue samples in different temperatures since our study is limited to 4 and –20°C. Using smaller volumes of fat tissue, such as the Coleman procedure, can increase tissue exposure to critical nutrients. Thus, further investigations are needed to determine the viability of different amounts of fat tissues.

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Ethical Approval

This study has been approved by the ethics committee of Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1401.423). Consent to participate in the study and consent to publish the results have been approved by all participants.

Conflict of Interests

The authors declare that they have no competing interests.

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