The Effect of Centrifuge Duration on Fat Graft Survival

Oguzhan Demirel[1,2] Percin Karakol[2] Mehmet Bozkurt[2] Mehmet Ibrahim Tuglu[3]

[1] Plastic, Rekonsturctive and Aesthetic Surgery Department, Istanbul Aydın University Faculty of Medicine, Istanbul, Turkey
[2] Department of Plastic, Reconstructive, and Aesthetic Surgery, Bagcılar Research and Training Hospital, Istanbul, Turkey
[3] Department of Histology-Embriyology, Celal Bayar University Medical Faculty, Manisa, Turkey

Address for correspondence Oguzhan Demirel, MD, Istanbul Aydın University Faculty of Medicine, Plastic, Rekonsturctive and Aesthetic Surgery Department, Florya Yerleşkesi (Halit Aydın Yerleşkesi), Beşyol Mah.Inönü Cad. No: 38, Sefaköy–Küçükçekmece/İSTANBUL, Turkey (e-mail: mdoguzhandemirel@gmail.com).

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Abstract

Background Although fat grafts are widely used for reconstruction and aesthetic purposes, their survival rates differ significantly. Centrifugation is one of the methods used to increase the survival of fat grafts. However, experimental studies examining the long-term outcomes of centrifugation duration are currently limited. Thus, in the present study, the effects of centrifugation duration on the survival of fat grafts were assessed using an animal model.

Methods Thirty Sprague Dawley rats were included in the study and fat grafts were obtained from each specimen by excision from inguinal fat pads. Preparation protocols were administered as an en-bloc fat graft in Group 1, minced fat graft in Group 2, and fat graft centrifuged at 1,054 ×g for 2 minutes, 3 minutes, and 4 minutes in Group 3, 4, and 5, respectively. After 12 weeks of follow-up, grafts were harvested and were subjected to histopathological evaluation based on an established scoring system.

Results En-block fat grafts were associated with necrosis, fibrosis, inflammation, vacuole formation, and alterations in adipocyte morphology. Among the three centrifugation groups, Group 3 demonstrated the best adipocyte viability and vascularity. However, graft weights decreased in all experimental groups.

Conclusion The centrifugation process may have positive effects on adipocyte survival by means of purifying the fat graft and increasing adipocyte concentration. When the centrifugal durations were compared, 3-minute centrifuge yielded the most favorable results.

Keywords
- adipose
- centrifugation
- graft
- duration
- survival

Introduction

Autologous fat graft (AFG) has been widely used in aesthetic and reconstructive surgery for various purposes for many years. Although non-immunogenic, easily harvested, low rates of infection, and cheap features make this technique gain popularity over years, unpredictable long-term results and need for second or more interventions were major drawbacks. A wide range of survival rates in the literature suggests that there is no gold standard technique in AFG.[1–3]

There were various parameters demonstrated to affect fat graft survival in different studies.[4–7] Thus, it is coherent to

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separate the fat grafting process step by step, and assess the impact of every parameter to achieve a standard method for AFG. Correspondingly, fat graft process can basically be evaluated in three main headings: Graft harvesting, graft preparation, and graft transfer to the recipient area. The aim of the graft preparation, as the most important step, is to purify the adipose tissue from fragments such as lipid, protease, cell debris, and blood cells.[8,9] For these purposes, decantation, washing, filtration, and centrifugation are the common techniques that exist in the literature.[3–5,8,9]

Among these techniques, centrifugation is one of the most widely used and discussed methods in literature. In many studies conducted by Coleman et al, it was suggested that the adipose layer could be effectively purified by separating it from liquid and lipid layers with 3,000 rpm centrifugation for 3 minutes.[2,10] Moreover, there are studies indicating that centrifugation causes adipocyte damage despite this decomposition effect.[11,12]

Therefore, further investigation of this technique is needed. When the literature was reviewed, many studies on centrifugal force and velocity (speed and velocity: rpm, force: g) were observed, whereas studies that assess the effect of centrifugation duration on fat graft survival were limited.[3,8,11,13]

Importantly lack of experimental long-term animal studies makes it difficult to obtain accurate data. Thus, the aim of this study is to investigate the effect of different centrifugal times under constant centrifugal force in the experimental rat study model for the following 3 months for long-term results.

Materials and Methods

This study was conducted under the permission of the Health Sciences University, Bagcılar Training and Research Hospital Local Ethical Committee. Thirty Sprague Dawley rats with an average weight of 250 to 300 gr were enrolled in the study.

Experimental Groups

Rats were divided into five groups according to the computer-generated randomization program. In group 1 (control group) en bloc fat grafts and in group 2 minced fat grafts were used. Centrifugation was not enrolled in these groups. Samples were centrifuged for 2 minutes in group 3, for 3 minutes in group 4, and for 4 minutes in group 5 with 2,500 rpm, 1,054 g , and 15.1 cm rotor radius (►**Table 1**).

Table 1 Experimental groups

Group 1 (Control)	**En bloc fat grafts**
Group 2	Minced fat grafts
Group 3	2 min centrifuge with 2,500 rpm/1,054 g
Group 4	3 min centrifuge with 2,500 rpm/1,054 g
Group 5	4 min centrifuge with 2,500 rpm/1,054 g

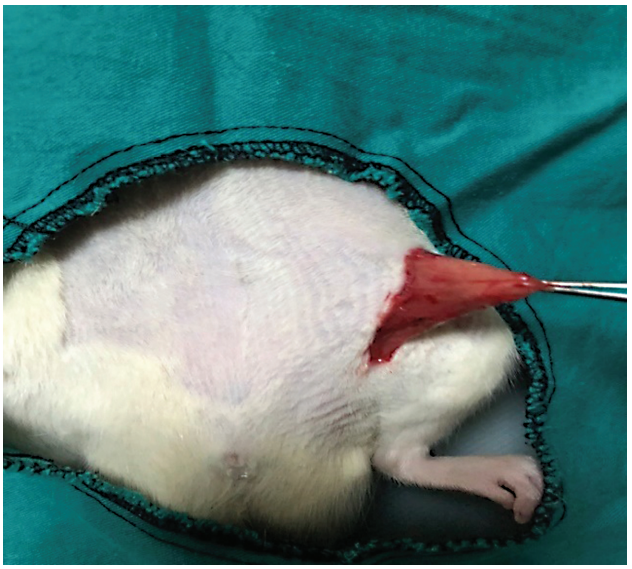

Fig. 1 Inguinal fat pad excision.

Surgical Technique

Harvest of the Graft

After the left inguinal area and parietal scalp areas of the rats were shaved in each group, the inguinal fat pads were dissected with the appropriate incision settled in the inguinal crisis. The fat pad was carefully excised and the skin was sutured (►**Fig. 1**).

Graft Preparation

0.9 gr fat grafts were obtained for each animal. In group 1, fat grafts were not processed and en bloc fat graft samples were obtained. In group 2, fat grafts were minced into small pieces (2–4 mm) via a scalpel. Fat grafts from groups 3 to 5 were centrifuged at 2,500 rpm and 1,054 g for 2, 3, and 4 minutes, respectively. After centrifugation, three layers were obtained and the upper lipid layer and the underlying liquid layer were isolated and discharged, and the middle adipose layer was isolated. Since the liposuction technique and tumescent solution were not used, it was observed that the oil and liquid layers obtained in the centrifuge groups were in trace amounts. Therefore, the injected graft weight was accepted as 0.9 gr for all subjects. GLO GT416 centrifuge instrument (Glofinn Ltd., Finland) was used for centrifugation. The rotor length of the centrifuge device used was 15.1 cm. Macroscopic views of fat grafts after preparation were mentioned in ►**Fig. 2**.

Fat Graft Transfer to the Recipient Area

An incision with 1 cm length was made in the parietal scalp area for group 1 and group 2. After the preparation of subcutaneous tissue plan, en bloc and minced fat graft were transplanted, respectively. Grafts were transferred in 1 mL syringes. In groups 3, 4, and 5, fat graft injections were performed with 18-gauge angiocatheter in subcutaneous plane with multiple directions. Bolus injection of fat grafts was avoided. Injections were made when cannula was withdrawn. 0.1 mL small fat aliquots were given with multiple passages (►**Fig. 3**).

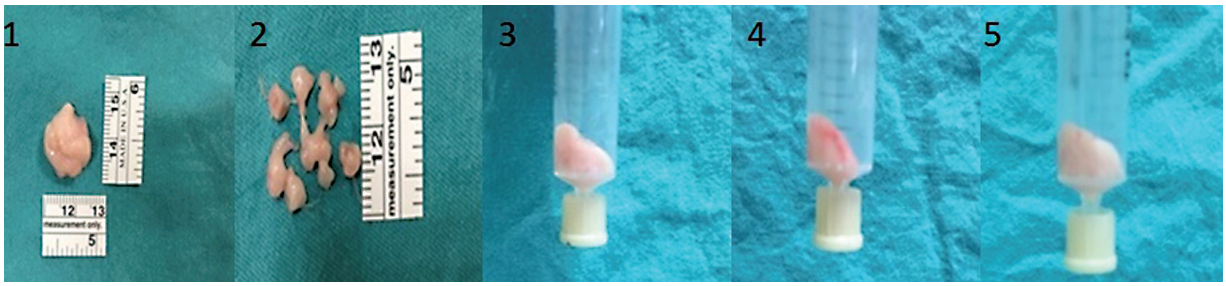

Fig. 2 Macroscopic views of fat grafts after preparation for each group.

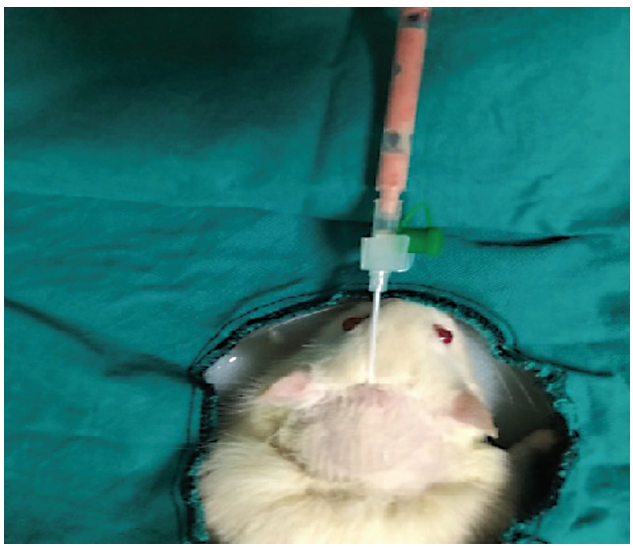

Fig. 3 Transfer of the fat graft to the recipient site with 18-gauge angiocatheter.

Re-operation

After 12 weeks of follow-up, the rats were taken to the prone position under anesthesia. Approximately 1 cm incisions were made on the scalp, the observed fat tissues were excised by the exclusion of the skin. The excised fat grafts were weighed. The samples were delivered to the histology laboratory in the cold chain.

One rat in groups 1 to 4 and two rats in group 2 were lost due to unknown reasons in the follow-up period. The results were obtained from five rats in group 1, four rats in group 2, five rats in group 3, five rats in group 4, and six rats in group 6.

Histopathological Analysis

Histopathological sections were prepared with hematoxylin-eosin (HE) staining at the Health Science University, Bağcılar Training and Research Hospital Pathology Department. The sections were delivered to the Department of Histology and Embryology at Celal Bayar University for histopathological examination.

A histopathological scoring system was performed in this study. Adipocyte viability, adipocyte shape, necrosis, vacuole formation, inflammation, fibrosis, mononuclear cell infiltration, and vascularization parameters were assessed with a light microscope under $\times 4$, $\times 10$, and $\times 40$ magnification. Each parameter was evaluated depending on the following scale: 0 = absence, 1 = minimal presence, 2 = minimal to moderate presence, 3 = moderate presence, 4 = moderate to extensive presence, and 5 = extensive presence.

Statistical Analysis

Data were analyzed using SPSS 11.0 (Chicago, Illnois, United States) statistical program. Results will be considered as significant when the *p*-value is <0.05. Non-parametric Kruskal–Wallis test and multiple-way Mann-Whitney U test for independent variables will be used for comparison of multiple groups.

Results

Fat grafts were excised and weighted after 3-month follow-up. Although graft weights were decreased in all samples no significant difference was observed between the groups (►**Table 2**).

Table 2 Graft weights (gr) after sacrification

Group number Rat number	Group 1	Group 2	Group 3	Group 4	Group 5
1	0.62	0.44	0.58	0.45	0.44
2	0.48	0.52	0.62	0.57	0.51
3	0.44	0.40	0.43	0.66	0.52
4	0.36	0.46	0.47	0.52	0.41
5	0.52		0.42	0.55	0.49
6					0.46
Mean weight (gr)	0.484	0.455	0.504	0.550	0.471

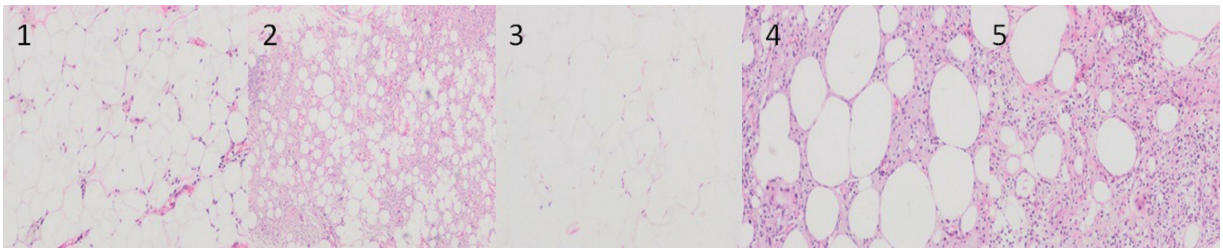

Fig. 4 Adipocyte necrosis under 10× light microscope images with hematoxylin-eosin staining.

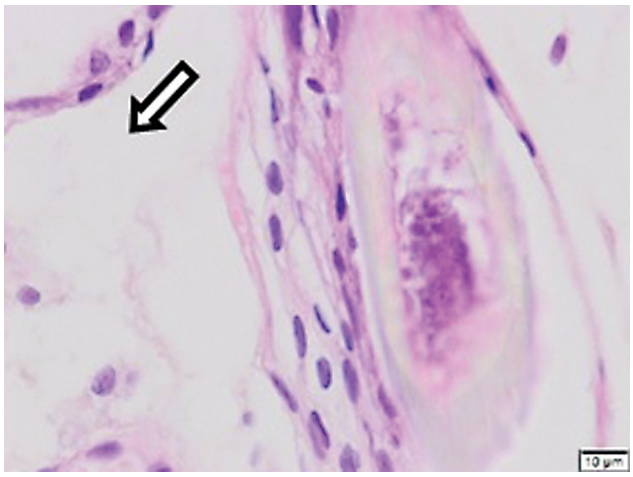

Fig. 5 Adipocyte necrosis features; adipocyte membrane loss, absent nucleus, cell fusion (*black arrow*).

HE-stained samples were evaluated for adipocyte viability, necrosis, vacuole formation, inflammation, fibrosis, mononuclear cell infiltration, and vascularization parameters.

Nucleated viable adipocytes with normal morphology were observed more frequently in centrifugation groups compared with group 1. However, necrotic areas, fat cysts, enlarged fat vacuoles were also detected in centrifugated samples. Pointedly, these viable cells were more commonly located in areas close to the periphery of the graft. Additionally, fibrotic areas were in wider regions and capillary blood vessels were more intense in adipocytes compared with the control group. When the necrosis, fibrosis, cyst formation, and inflammation were evaluated, the highest values belonged to group 1 (►**Fig. 4**). Necrosis features were mentioned in ►**Fig. 5**.

The mean necrosis score was lower in group 2 and group 4 (mean values were 1.750 ± 0.758 and 1.667 ± 0.605, respectively). However, the highest amount of necrosis was detected in group 5 (►**Table 3**). There was no statistically significant difference between the groups ($p = 0.3216$).

Adipocytes were evaluated depending on their viability and adipocyte morphology (►**Fig. 6**). Viable adipocytes contain peripheral situated nucleus and intact membrane (►**Fig. 7**).

The highest adipocyte viability was observed in group 4, and it was at the lowest level in group 1. There was no statistically significant difference between group 1 and group 2 ($p > 0.05$) whereas there was significant differences between group 1 and group 3 ($p < 0.05$), and group 4 ($p < 0.001$) and group 5 ($p < 0.01$). When the group 2 and other groups were compared, the only significant difference was observed for the group 4 ($p < 0.01$). No significant difference was detected between the centrifugation groups. According to adipocyte shape differences, despite group 2 and group 4 samples had similar characteristics, there were no statistically significant differences discovered between the groups ($p = 0.3922$) (►**Tables 3** and **4**).

In the evaluation of fibrosis and mononuclear cell infiltration, higher scores were observed in group 1 and group 5. Thick collagen wire bundles and fibroblasts were the main features in fibrosis areas. Moreover, the lowest scores were detected in group 2 and group 4. However, no statistically significant difference was obtained between the groups.

In terms of vascularity parameters, the number of capillary blood vessels was evaluated. Similarly poorer results were observed in group 1 (►**Table 5**). While there was a statistically significant difference between group 1 and group 4, and group 4 and group 5 ($p < 0{,}01$ and $p < 0.05$, respectively), no significant difference was detected between

Table 3 Mean scores of histopathologic parameters in each group

Groups	Histopathologic parameters					
	Viability	Necrosis	Vascularity	Morphology	Fibrosis	Mononuclear cell infiltration
G1	2.000	2.083	1.583	3.083	2.667	3.655
G2	2.000	1.750	2.583	2.833	2.333	2.575
G3	3.000	2.167	2.583	3.333	2.750	3.650
G4	4.000	1.667	3.417	2.833	2.583	3.030
G5	3.250	2.500	2.000	3.417	3.417	3.865

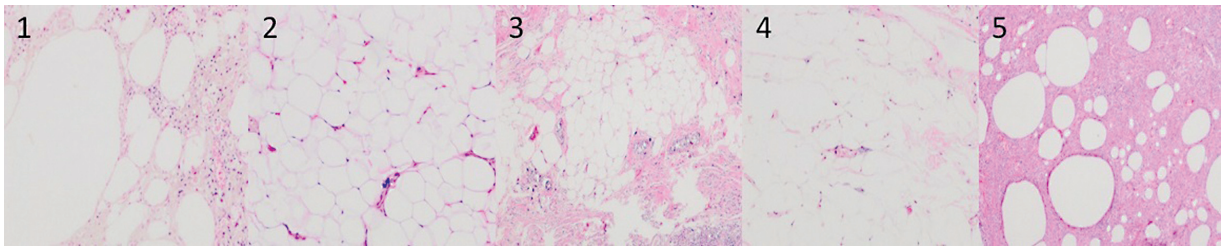

Fig. 6 Adipocyte viability under 40× light microscope images with hematoxylin-eosin staining.

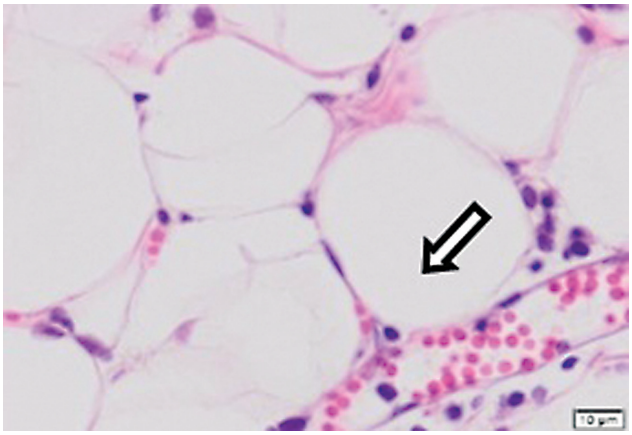

Fig. 7 Viable adipocytes with peripherally situated nucleus and intact cell membrane (*black arrow*).

Table 4 Statistical results after comparison of viability between groups

Groups	Differences	*Q*-value	*p*-Value
G1 vs. G2	−0.3333	1.348	$p > 0.05$
G1 vs. G3	−1.250	5.056	$p < 0.05$
G1 vs. G4	−1.833	7.416	$p < 0.001$
G1 vs. G5	−1.333	5.394	$p < 0.01$
G2 vs. G3	−0.9167	3.708	$p > 0.05$
G2 vs. G4	−1.000	6.068	$p < 0.01$
G2 vs. G5	−1.500	4.045	$p > 0.05$
G3 vs. G4	−0.5833	2.360	$p > 0.05$
G3 vs. G5	−0.08333	0.3371	$p > 0.05$
G4 vs. G5	−0.5000	2.023	$p > 0.05$

Table 5 Statistical comparison of vascularity between groups

Groups	Mean	Deviation	Median
G1	1.583	0.6646	1.500
G2	2.583	0.7360	2.750
G3	2.583	0.8070	2.250
G4	3.417	0.8010	3.750
G5	2.000	0.9487	2.000

the other groups. Under light microscopic observations conducted for neovascularization, a few capillary formations were observed in groups.

Discussion

There are many studies on fat graft survival because of the increasing popularity of autologous fat grafting in the clinic setting. However, mechanical factors on adipocytes during the harvesting, processing (purification), and transfer of fat graft to the donor area affect fat graft survival.[4,5,12]

The fat graft purification (centrifugation, decantation, washing, filtration, etc.) process maintains its importance to achieve better fat graft survival. Among fat purification techniques, centrifugation is noteworthy and widely used.[2,8,10]

When the literature is examined, centrifugation protocol, which was 3,000 rpm and 1,286 g and performed for 3 minutes, as defined by Coleman, had high rates and was accepted by other authors. This technique claimed that adipocyte viability was maintained via effective separation of fat graft from lipid, protease, blood cells, and cellular debris.[2,14,15]

However, there are different results in the literature. In a study, it was stated that centrifugal force up to 20,000 gauge did not increase apoptosis, and cell damage compared with low centrifugation or non-centrifuged groups.[16] Similarly, it has been reported that the structure of the fat graft, centrifuged at 5,000 g, has the healthiest histological values.[17] However, in a study conducted by Piasecki et al, 228 g and in a study by Boschert et al, 50 g centrifugation force was the most effective.[18,19]

This wide range of differences can be caused by misuse of two different parameters: centrifugal force (g force) and centrifuge speed (rpm: revolutions per minute). When the formula is examined, the g force is not only related to rpm, but also related to the rotor length (rotor radius, cm) in the centrifugal device used [g force = 1,118 × rotor length (rotor radius, cm) × rpm 2 × 10–9]. The increase in rotor length will increase the g force. This suggests that if a study is performed and the rotor length is not mentioned in that study, the idea of the selection of g force in the methodology would be more effective in terms of standardization, rather than rpm.

As an example, in a study conducted by Kim et al, 3,000 rpm centrifugal speed with 3 minutes was recommended.[20] Rotor length (22 cm) was given in the study and

3,000 rpm corresponds to approximately 2,213 g after all calculations made. Compared with the Coleman technique, the rpm that was equal to the g force was totally different.[2,10,14]

When studies are assessed based on rotor, rpm, and g force parameters, the result indicating that centrifugal forces stronger than 1,286 g may cause adipocyte damage can be obtained.[3,11,13] Thus, in our study, 2,500 rpm with 1,046 g protocol was chosen by using the centrifugal device under clinical conditions.

As there are different results in the literature about centrifuge speed, there is a similar situation with regard to the duration of centrifugation. Considering the abundance of studies on centrifugal speed, the minority of experimental studies investigating centrifugation time is remarkable. Although 3 minutes centrifuge protocol which was defined by Coleman is widely used, the vast majority of the studies conducted by Coleman are observational clinical studies.[2,10,14,21] Therefore, as the main purpose of our study, in vivo controlled animal studies are needed to achieve more accurate assessment and to reveal long-term results.

According to the literature assessment, there is a limited experimental study that addresses the effect of centrifugation duration on fat graft survival (►**Table 6**). Also, methodological differences between these studies prevent reaching common data.[3,13,18–20]

In an experimental study by Hoareau et al 100 g/1 second, 100 g/1 minute, 400 g/1 minute, 900 g/1 minute, 900 g/3 minutes, and 1,800 g/10 minutes centrifugation protocols were assessed.[3] Although the study was attempted to investigate force and time, there were no comparative groups for the proposed 400 g/1 minute centrifuge protocol at the end of the study. However, adipocyte viability was measured indirectly (amount of lipid released) and histopathological evaluation was performed with HE. Moreover, the 4-week follow-up period is insufficient to demonstrate long-term results.

In a study by Ferraro et al, different centrifugal speeds and centrifugation times were utilized (500 rpm [50 g] + 10 minutes, 1,300 rpm [250 g] + 5 minutes, and 3,000 rpm [1,500 g] + 3 minutes). The study was performed both in vitro and in vivo. At the end of the study, 5 minutes of centrifugation protocol with 250 g was proposed.[13] Although suggestions were made based on centrifugation time in the discussion section, controlled experimental groups (different centrifugation speeds were used in each group) were not established to investigate centrifugation duration.

In another study by Kim et al 1,500 rpm for 1/3/5 minutes, 3,000 rpm for 1/3/5 minutes, and 5,000 rpm for 1/3/5 minutes centrifugation protocols were assessed. High cellular damage was detected especially in the centrifuge groups at 5,000 rpm and for 5 minutes at 1,500 rpm. In the study, it was asserted to limit the speed to a maximum of 3,000 rpm and a maximum duration of 5 minutes. Also, a centrifugation protocol with 3,000 rpm and 3 minutes was proposed.[20] There are some noteworthy methodological differences in this study. First, after centrifugation each tube was shaken again by gentle rinsing and the adipose layer was isolated after being left to decant for 5 minutes. In this case, it can be considered that decantation may affect the results. At the same time, according to the centrifugation features of rotor length of 22 cm, 3,000 rpm and 3 minutes, the centrifugation force corresponded to 2,213 g. It was relatively high when compared with the Coleman technique and the literature. As an important difference from our study, since it is planned in vitro, it does not provide long-term results.

Also, Piasecki et al investigated centrifuge duration with the experimental groups of 228 g (1,000 rpm) for 1, 2, 3, 5, and 10 minutes. The results revealed that there was no additional contribution to purity and viability after 3 minutes. When the methodology was examined, collagenase treatment was performed after centrifugation. Similarly, it did not reflect long-term results because of in vitro study design.[18]

In vitro study conducted by Boschert et al was performed with 50 g centrifugation for 2, 4, 6, and 8 minutes, and it was observed that there was no additional contribution in the centrifugation time after 2 minutes.[19]

When considering these experimental studies, only one of these was an in vivo study. As mentioned above, the 4 weeks follow-up duration and lack of controlled experimental groups were the major drawbacks of this in vivo study.[3] Also methodological differences in other in vitro studies such graft harvest technique, different centrifugation force, and fat graft survival assessment techniques make it difficult to compare data. Furthermore, the major disadvantage of these studies is that they do not provide information on long-term survival.[13,18–20]

To achieve more accurate and comparable results, every steps affects fat graft survival take in consideration in our study. First, when mechanical factors are assessed, graft harvest may be taken through excision or liposuction. However, the effect of these methods on graft survival has been investigated in many studies, it has been stated that the excision method is associated with better survival rates, morphology, and higher stem cell concentration.[22–25]

Since the elimination of variables like liposuction type, negative pressure, cannula size, number of holes, and superficial structure of cannula, the excision technique seemed to be more suitable for the aim of the research. Therefore, direct excision method was selected for the graft harvest in this study.

In general, it is thought that the 3-minute centrifugation time recommended by Coleman will effectively separate the fat graft into layers.[4,9,14,21,26] Therefore, to be comparable with the literature, 2 minutes, 3 minutes, and 4 minutes centrifugation durations were included in our study.

However, another important factor that may affect the outcomes of the study is fat graft transfer to the recipient area. In this regard, the amount of the fat graft given in each passage, the speed of delivery, and the size of the cannula are important. Also, the size of each adipocyte cluster in the fat graft and the distance to the capillary network have been demonstrated to be very important in adipocyte viability.[2,27]

Table 6 Study information that investigate centrifuge duration

Author	Methodology	Groups	Centrifugation features	Assays	Recommended centrifugation time	Recommended centrifugation force/speed
Boschert	In vitro Fat source: Human fat Fat harvest: Liposuction	G1: 2 min G2:4 min G3:6 min G4:8 min	Rotor length: 17.2 cm Rpm: not given g force: 50 g	Trypan Blue staining	2 min	50 gauge
Piasecki	In vıtro Fat source: Mouse inguinal fat Harvesting technique: direct	G1:1 min G2:2 min G3:3 min G4:5 min G5:10 min	Rotor length: not given Rpm: 1,000 rpm g force: 228 g	Trypan Blue staining	3 min	228 gauge
Kim	In vitro Fat source: Human fat Fat harvest: Liposuction	G1: 1 min G2: 3 min G3: 5 min G4:1 min G5: 3 min G6: 5 min G7: 1 min G8: 3 min G9: 5 min	Rotor length: 22 cm Rpm: G1/G2/G3: 1,500 rpm G4/G5/G6: 3,000 rpm G7/G8/G9: 5,000 rpm g force:not given	Trypan Blue staining	3 min	3,000 rpm
Hoareau	In vitro/In vivo Fat source: Human fat Fat harvest: Liposuction Fat recipient: Mouse Duration: 1 mo	G1: 0 G2: 100 g G3: 100 g G4: 400 g G5: 900 g G6: 900 g G7: 1,800 g	Rotor length: not given RPM: not given Centrifuge duration: G1: 1 s/G2: 1 min/G3: 1 min G4: 1 min/G5: 1 min /G6: 3 min G7: 10 min	Masson trichrome staining Serum IL-6, MCP-1 level ELISA	1 min	400 gauge
Ferraro	In vitro Fat source: Human fat Fat harvest: Liposuction	G1: 500 rpm/50 g G2: 1,300 rpm/250 g G3: 3,000 rpm/1,500 g	Rotor length: not given Centrifuge duration: G1: 10 min G2: 5 min G3: 3 min	Cell culture Flow cytometry Apoptosis assays Hematoxylin-eosin staining Mallory's trichrome staining Light microscopy Immunofluorescence Immunohistochemistry	5 min	1,300 rpm
	In vivo Fat source: Human fat Fat harvest: Liposuction Fat recipient: human	G1:control (decantation) G2: 1,300 rpm/250 g G3: 3,000 rpm/1,500 g	Rotor length: not given Centrifuge duration: G1:10 min G2: 5 min G3: 3 min	Photography	5 min	1,300 rpm

In a study performed by Carpaneda and Riberio, interactions between the fat graft and the recipient area were investigated, and they suggested that the diameter of the graft should be 3 mm due to the fact that the diffusion was only up to 1.5 mm apart all margins.[28] Similar results have been reported in many other studies.[29,30]

In our study, the fat graft which was transplanted in group 2 was divided into 2 to 3 mm pieces. The injection was performed in different plans, the cannula is retracted and controlled to give 0.1 mL each time. Fat injections performed at groups 3, 4, 5 were done with 18-gauge angiocatheter. When the literature was examined, it was concluded that larger cannulas (16, 18, 20 gauge) could appear with more successful results, but also good results were obtained with narrow diameter cannulas like 22 gauge and 24 gauge.[7,18,25]

Based on our results, it was evaluated that the 3 minutes was associated with better results in all parameters, and this data was determined to be consistent with the literature.[10,14] Furthermore, there are some limitations of the following study. When the literature was examined despite of several rat studies, most of the experimental studies were performed on mice. Similarly, liposuction was performed commonly for graft harvest.[2,3,31] As mentioned above, excision method was selected in our study to eliminate parameters about fat harvest step and evaluate directly the effect of centrifugation. In addition, since the liposuction method and tumescent solution were not used, it was observed that the oil and liquid layers were in trace amounts after the centrifugation process. Therefore, the graft volume lost after centrifugation was not calculated specifically. In addition, increasing the number of samples may provide statistically more advanced results.

Also, despite HE dye is widely used in many studies, it cannot give information about adipocyte counts. Additionally, there could be a failure to differentiate the dead and viable adipocytes (round adipocytes in the form of round lipid droplets can be easily confused with live adipocytes). The use of different immunohistochemical or colorimetric assays for evaluation may increase the reliability of the results.

The assessment of long-term effects of the centrifugation time on the fat graft with an experimental animal study, the creation of the methodology in consideration of the literature suggestions in each step, and the detailed consideration of the parameters in each step that affect fat graft survival were important characteristics of the following study.

Conclusion

The graft preparation techniques prior to the recipient area transfer is of great importance for graft survival. As one of the methods of purification, centrifugation, speed and time variables prevent the establishment of a standard protocol. Although there are many studies on the centrifugation speed or force in the literature, studies on the centrifugation time are limited. In our study, we suggest that 3 minutes of centrifugal force at 1,054 g is effective in the fat graft, and is associated with increased adipocyte viability and vascularity. Further studies with the careful investigation of each parameter that affects the survival of the fat graft and reveal long-term results can provide further information on this subject.

Ethical Approval

This study was conducted under the permission of the Health Sciences University Bagcılar Training and Research Hospital Local Ethical Committee. All applicable international, national, and institutional guidelines for the care and use of animals were followed.

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Conflict of Interest

None declared.

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