Original Article / Özgün Araştırma

Fat Tissue Transfer: Is It Useful? *In Vitro* Study

Yağ Doku Transferi Yararlı Mıdır? İn Vitro Çalışma

Nazan Şahin Sivrioğlu[1]*, Hacer Banu Aksoy[2], Haşim Eray Copcu[3]

[1]Aydın Adnan Menderes University Faculty of Medicine, Department of Plastic and Recontructive Surgery, Aydın, Turkey
[2]Private Specialist in Plastic and Recontructive Surgery, İstanbul, Turkey
[3]Private Specialist in Plastic and Recontructive Surgery, İzmir, Turkey
*We regret to inform you that Prof. MD., Nazan Şahin Sivrioğlu has passed away.

Abstract

Objective: This study aimed to establish *in vivo* adipose tissue, autologous transplantation of preadipocytes and adipocytes amplified in rat adipose culture suspensions and to compare histological features, adipose tissue formation and permanent volume following autologous transfer.

Materials and Methods: There were 32 rats in the study divided into 4 groups. Adipocyte cell suspensions were injected into the scapular and evaluated 4, 8, 12, and 24 weeks later for groups I, II, III, and IV, respectively. Histological and volumetric changes were detected.

Results: Although fatty tissue volume increased with time, there was no significant difference was between the groups. Histological scores were not different between groups ($p>0.05$).

Conclusion: In our study, the formation of fatty tissue in healthy appearance was observed in a similar manner after autologous transplantation of the preadipocytes and adipocyte cells in the culture. In the future, we believe that the preadipocyte cell suspension can be transplanted in cell culture and can be transferred autologous to fill the soft tissue defect in the same patient.

Keywords
Adipocyte cell culture, autologous tissue transfer, pre-adipocyte cell culture

Anahtar Kelimeler
Adiposit hücre kültürü, otolog doku transferi, preadiposit hücre kültürü

Received/Geliş Tarihi : 10.04.2019
Accepted/Kabul Tarihi : 26.06.2019

doi:10.4274/meandros.galenos.2019.88700

Address for Correspondence/Yazışma Adresi:
Nazan Şahin Sivrioğlu Prof. MD,
Hacer Banu Aksoy MD,
Private Specialist in Plastic and Recontructive Surgery, İstanbul, Turkey
Phone : +90 505 220 39 83
E-mail : drbanuaksoy@gmail.com

ORCID ID: orcid.org/0000-0003-3727-4008

Öz

Amaç: Çalışmanın amacı *in vivo* adipöz dokunun otolog transplantasyon, rat modelinde preadipositlerin ve adipositlerin kültürünün yapılması yoluyla güçlendirilmesini amaçlamaktadır. Otolog transfer sonrası histolojik özellikler, yağ dokusu formasyonu ve kalıcı volüm değerlendirilmiştir.

Gereç ve Yöntemler: Otuz iki rat eşit olarak dört gruba ayrıldı. Adiposit hücre süspansiyonu skapuler bölgeye enjekte edildi ve gruplar için sırasıyla 4, 8, 12 ve 24 hafta sonra histolojik ve volümetrik ölçümler yapılarak değerlendirildi.

Bulgular: Yağlı doku hacmi zamanla artmıştır, ancak gruplar arasında anlamlı bir fark bulunmadı. Histoloji değerlendirmesi sonucunda da fark saptanmadı ($p>0,05$).

Sonuç: Bu çalışmada preadipositlerin ve adipositlerin kültürünün yapılması sonrasında sağlıklı yağlı dokunun geliştiği gözlendi. Gelecekte, preadiposit hücre süspansiyonunun hücre kültürü sonrasında otolog doku transferi için hastalarda yararlı olabileceği görüşündeyiz.

Introduction

The use of fat grafting to instigate changes in body structure was a new procedure in the 20th century. Neuber (1) reported first individual fat graft in 1893 (2). Lexer (3) demonstrated the use of autogenous fat from abdomen to fill malar and zygomatic arch depression (4). Autologous fat transfer decreased until the procedure re-emerged in the late 80s. It is linked to the application of suction lipectomy commonly used in body shaping (5).

The goal of autogenous fat transfer includes the achievement of precisely controlled and noticeable change in contour with viable cells to maintain this improvement over the patient's lifetime with a decreased complication rate.

For this study, an animal model was designed to determine the cell type that would contribute most to the formation of adipose tissue with most persistence.

The aim of this study was to establish *in vivo* adipose tissue, autologous transplantation of pre-adipocytes and adipocytes amplified in rat adipose culture suspensions and to compare. histological features, adipose tissue formation and permanent volume following autologous transfer.

Materials and Methods

Adnan Menderes University Animal Experiments Local Ethics Committee approval was obtained for this study (approval number: 045, date: 25.09.2008). In our study, we used primary culture protocol of adipocyte precursor cells described by Vanessa van (6). A total of 32 adult male Wistar albino rats (350-450 gr) were used.

Experimental Design

The study consists mainly of five stages;

Stage I: Preparation of culture mediums to be used in the cell culture protocol.

Stage II: Taking fat tissue samples from rats.

Stage III: Passing through various operations in the cell culture protocol of fatty tissue samples. Isolating, reproducing and differentiation of a portion of the cells in the adipocytes of the precursor fat cells.

Stage IV: Autologous implantation of the pre-adipocytes and adipocytes cell suspensions replicated in the culture environment.

Stage V: Evaluation of injection sites periodically.

Preparation of Culture Mediums

Cell culture media, chemicals and culture materials were obtained from Sigma (Sigma, St. Louis, MO), Serva (Serva, Heidelberg, Germany) and Biochrom (Biochrom AG, Berlin, Germany).

Stage I

Basal media: Dulbecco modified eagle medium and Ham's F-12 solution were mixed equally in volume. 15 mmol (mM) 4-(2-Hydroxy Ethyl)-1-Piperazine ethane sulfonic acid, 14 mM $NaHCO_3$, 33 µmol (µM) biotin and 17 µM D-pantotenat were added. The mixture was filtered and the PH value was set to 7.4.

Saline solution with phosphate-buffered (PBS): The form of a tablet shaped solution was made according to the manufacturer's recommendations.

Collagenase solution: Collagenase (0.251 U/mg), %2 bovine serum albumin and PBS are prepared with pH 7.4.

Erythrocyte lysis buffer*:* 155 mM NH_4Cl, 5.7 mM K_2HPO_4, 0.1 mM ethylene diamine tetra acetic aside are mixed. Sterilized by filtration.

Inoculation media: The basal media was prepared with the addition of gentamicin (50 mg/mL) and 10% fetal beef serum.

Basal pre-adipocyte culture media: First, insulin, Tri-iodo-l-thyroxin and transfer of stock solutions were prepared. Tri-iodo-l-tyrosin 1 mM solution, 1 mol (m) was alkalised with NaOH, and in the 50% concentration of ethylene hydroxide, 2 µM was diluted into the stock solution.

Twenty-two µM insulin for insulin stock solution was created within 10 MM HCl. Transferrin stock solution was prepared within 1 mg/mL distillate water. The stock solutions were sterilized by filtration. In the basal media, the basal preadipocyte culture medium was created by adding insulin (66 nm), tri-iodo-l-thyroine (1 nM), transfer (10 µg/mL) and gentamicin (50 µg/mL) from the prepared stock solutions.

Preadipocyte differentiation media: First, Isobutyl-Methylxsantine (IBMX), hydrocortisone and troglitazon stock solutions were prepared. IBMX stock solution; 20 mM IBMX and Na_2CO_3 were alkalised. The 0.1 mM hydrocortisone stock solution was prepared in the ethylene hydroxide in 50%. The Troglitazone stock solution was prepared in dimethylsulfoxide, which is 1 mg/mL. With the addition of 0.5 mm IBMX, hydrocortisone (100 nM) and troglitazon (1 µg/

mL) to the basal preadipocyte culture medium, the preadipocyte differensiation medium was obtained.

Stage II

The study created 4 groups of 8 rats in each group, with a total of 32 adult male Wistar albino rats (350-450 gr weight). All rats had intramuscular 5 mg/kg xylasine applied and were put to sleep with 50 mg/kg ketamine injection.

By entering approximately 2 centimeters of vertical incision on the abdominal skin, the right epididymal region was reached with dissection and 2 grams of fat tissue was obtained from the fat pillow in the periepididymal region.

Stage III

After cell isolation, a sample of 20 µl was taken from the cell suspension and was suspended with an equal amount of Trypan blue, and the 10 µl of the mixture was transferred to thoma, and the x100 magnification was counted as live cells in a large square. After the cell count, the cell suspensions prepared from the fat samples of each rat were divided into two groups, with 1 million cells/mL for the production of preadipocytes and adipocytes.

The cells were transferred to the culture records to be the inoculation density 30,000-50,000 cell/cm^2. About 10 mL inoculation medium was added. For optimal cell adhesion; the cells were incubating at 37 °C in the incubation medium for 16-24 hours with a 5% CO2 rate in the incubator.

Basal preadipocyte culture medium was added to the environment for the production of preadipocytes in first group culture records. The preadipocyte differential media was added to the environment to provide the preadipocyte differentiation media in the second group of cultural plaques.

$10x10^5$ cell, were suspended with PBS, and 1 mL of cell suspensions were prepared for injection.

Stage IV

In the injection of cell suspensions, the animal model developed by Marler et al. (7) was referenced. Second anaesthesia was given to the rats, and under sterile conditions, the scapula regions were shaved bilaterally. 1 mL preadipocytes cell suspension was gently injected in the right and left scapula region.

According to the evaluation of the animals after the injection, 8 rats were divided into four groups in each group:

- 1st group: This group was evaluated 4 weeks after injection (n=8).

- 2nd group: This group was evaluated 8 weeks after injection (n=8).

- 3rd group: This group was evaluated 12 weeks after injection (n=8).

- 4th group: This group was evaluated 24 weeks after injection (n=8).

Stage V

First, the right scapular area was evaluated in the preadipocyte cell injection site in the region and the implants were reached in the gap between panniculus carnosus and paralumbar fascia and the visible white-fat tissue formations were excised. Excised material was evaluated by histo-pathology (Table 1).

Statistical Analysis

The data was analyzed with Kruskal-Wallis ANOVA and Dunn's multiple comparison post-test. Statistical analyses were performed using SPSS software, version 22.0 (IBM Corporation, Armonk, New York, United States). P<0.05 value was accepted as statistically significant.

Results

Volume Evaluation

Although the volume of the resulting fat samples increases by time, there is no statistically significant difference between the groups (p>0.05) (Table 2,3).

Table 1. Evaluation score system by histopathology

Criteria	Score		
	0	1	2
Necrosis	None	Low	Enlarged
Fibrosis	None	Low	Enlarged
Vascularity	High	Normal	Low
Cyst appearance	None	Microcyst	Macrocyst
Intensity of mast cell	Low (0-10 cell)	Normal (11-20 cell)	High (>20 cell)

Table 2. Preadiposit-volume changes by time

	Mean ± SD (cm^3)	Median (cm^3)	Minimum-maximum (cm^3)
Group I	0.67±0.16	0.66	0.42-0.89
Group II	0.70±0.27	0.63	0.45-1.32
Group III	0.76±0.07	0.76	0.66-0.88
Group IV	0.88±0.52	0.68	0.43-2.10

SD: Standard deviation

Histopathologic Evaluation

Compared to the total scores; although the average total scores had increased among the groups by the evaluation time, there was no statistically significant difference between the groups (p>0.05) (Table 3,4).

Table 3. Preadipocyte-Histopathologic scores evaluation

	Mean ± SD	Median	Minimum-maximum
Group I	2.71±1.50	3.0	1.0-5.0
Group II	2.88±0.64	3.0	2.0-4.0
Group III	3.38±0.92	3.0	2.0-5.0
Group IV	3.50±1.60	4.0	1.0-5.0
SD: Standard deviation			

Table 4. Adipocyte-histopathologic scores evaluation

Group	Mean ± SD	Median	Minimum-maximum
Group I	4.71±0.76	5.0	4.0-6.0
Group II	4.75±0.89	4.5	4.0-6.0
Group III	4.75±1.28	4.5	3.0-7.0
Group IV	5.25±1.28	5.0	4.0-7.0
SD: Standard deviation			

Discussion

In our study, fatty tissue formation with healthy appearance was observed in similar ways after autologous transplantation of preadipocyte and adipocyte cells in the culture. The preadipocyte injected 1 mL cell suspension volume was noted to retain an average of 88% at the end of 6 months. In the adipocyte injection group, a significant decrease in volume was observed in the early period and only 38% of the volume injected at the end of 6 months could be preserved. It was revealed that the adipocyte differencing (AD) of the preadipocytes *in vivo* was successful. The resulting hypoxic environment in the early period after transplantation was more durable than the adipocytes and the volume of the fatty tissue obtained was more stable.

Torio-Padron et al. (8) showed that in the first 3-4 weeks, progressive graft absorption is observed and then the implant volume and its shape remains constant. Their experiment showed, under microscopic examination, the presence of well-organized fat tissue, even at the end of 6 months. Inflammatory response, tissue necrosis and cyst formation were not detected. The new tissue was stable for nine months. As the concentration of cells increased, the formation of fatty tissue was detected.

Some researchers argue that the provision of *in vitro* AD of preimplantation preadipocytes will increase adipose tissue regeneration (9). For this reason, the preadipocytes were reproduced on synthetic or natural polymer scaffolds and *in vivo* adipose tissue was attempted to be created with the implant. However, although new tissues were formed, AD was not satisfactory (10).

Cho et al. (9) demonstrated a reduction of at least 30% in the volume of implants in all groups at the end of the sixth week. The preadipocyte implantation, conducted with AD, increased vascularity and *in vivo* adipogenesis in comparison to the differentiation of preadipocytes. One of the most important components of the histopathologic scoring system we developed is the evaluation of vascularity intensity. In groups, which were given the culture of preadipocyte injections, a significant increase in the vascularity was observed in all groups. It is thought that when the preadipocytes are exposed to ischemia, it increases vascularization by secreting angiogenic factors and stimulating growth factors (11). In the adipocyte injection group in the culture, the first group has a significantly less vein density and at the end of the 6th month, an increase in the formation of new veins was noted. We believe that failure to create adequate vascular support following the ischemic environment initially exposed by adipocytes, is responsible for a significant reduction in the fat tissue volumes obtained from the first groups.

Conclusion

In our study, the formation of fatty tissue in healthy appearance was observed in similar ways after autologous transplantation of the preadipocytes and adipocytes cells in the culture. In the future, we believe that the preadipocyte cell suspension can be transplanted in cell culture and can be transferred as autologous to fill the soft tissue defect in the same patient.

Acknowledgement

This article is dedicated to the memory of our dear colleague Nazan Şahin Sivrioglu Prof. MD, passed away while the article was in the publication process.

Ethics

Ethics Committee Approval: Adnan Menderes University Animal Experiments Local Ethics Committee approval was obtained for this study (approval number: 045, date: 25.09.2008).

Informed Consent: Informed cnsent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: N.Ş.S., H.B.A., H.E.C., Concept: N.Ş.S., H.B.A., H.E.C., Design: N.Ş.S., H.B.A., H.E.C., Data Collection or Processing: N.Ş.S., H.B.A., H.E.C., Analysis or Interpretation: N.Ş.S., H.B.A., H.E.C., Literature Search: N.Ş.S., H.B.A., H.E.C., Writing: N.Ş.S.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Neuber GA. Fett Transplantation. Verl Dtsch Ges Chir 1893; 22: 66.
2. Billings E Jr, May JW Jr. Historical review and present status of free fat graft autotransplantation in plastic and reconstructive surgery. Plast Reconstr Surg 1989; 83: 368-81.
3. Lexer E. Freie fett transplantation. Dtsch MEd Wochenschr 1910; 36: 640.
4. Hinderer UT, del Rio JL. Erich Lexer's mammaplasty. Aesthetic Plast Surg 1992; 16: 101-7.
5. Kaufman MR, Bradley JP, Dickinson B, Heller JB, Wasson K, O'Hara C, et al. Autologous fat transfer national consensus survey: trends in techniques for harvest, preparation, and application, and perception of short- and long-term results. Plast Reconstr Surg 2007; 119: 323-31.
6. Vanessa van H, Skurk T, Hauner H. Primary culture and differentiation of human adipocyte precursor cells. Human cell culture protocols. Humana Press 2005: 125-35.
7. Marler JJ, Guha A, Rowley J, Koka R, Mooney D, Upton J, et al. Soft-tissue augmentation with injectable alginate and syngeneic fibroblasts. Plast Reconstr Surg 2000; 105: 2049-58.
8. Torio-Padron N, Baerlecken N, Momeni A, Stark GB, Borges J. Engineering of adipose tissue by injection of human preadipocytes in fibrin. Aesthetic Plast Surg 2007; 31: 285-93.
9. Cho SW, Kim I, Kim SH, Rhie JW, Choi CY, Kim BS. Enhancement of adipose tissue formation by implantation of adipogenic-differentiated preadipocytes. Biochem Biophys Res Commun 2006; 345: 588-94.
10. Hemmrich K, von HD, Rendchen R, Di BC, Milella E, Pallua N. Implantation of preadipocyte-loaded hyaluronic acid-based scaffolds into nude mice to evaluate potential for soft tissue engineering. Biomaterials 2005; 26: 7025-37.
11. Yoshimura K, Suga H, Eto H. Adipose-derived stem/progenitor cells: roles in adipose tissue remodeling and potential use for soft tissue augmentation. Regen Med 2009; 4: 265-73.