

Comparison of five methods of fat grafting

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BACKGROUND: Recently autologous fat tissue transfer has gained much popularity. On the other hand, applying the transferring methods with a higher survival rate is still controversial. The aim of this study is to compare dermis-fat graft and dermal-fascia-fat graft. **METHODS:** This animal study was conducted in 2008-2009. 12 dogs underwent fat graft transfer in forms of free fat, dermis-fat and dermal-fascia-fat. Two months post operation the grafts were removed and analyzed microscopically and macroscopically. **RESULTS:** The most significant weight loss was observed in circular free fat while, dermal-fascia-fat graft had the least. According to microscopical analysis the most considerable angiogenesis was improved in dermal-fascia-fat graft. **CONCLUSIONS:** Dermis-fat graft had a higher survival rate than free fat graft. Considering the higher angiogenesis and collagen synthesis used in the dermal-fascia-fat group, it seems that fat graft has more maintenance in the presence of fascia. To determine the influences of fascia further studies are recommended.

KEYWORDS: Fat Transfer, Dermis-fat-graft, Dermis-fascia-fat, Contour Defect

BACKGROUND

Recently, fillers have been considered as important elements in restoring contour defects. There are several artificial substances available to fill subcutaneous defects such as collagen, hyaluronic acid, silicon, methyl acrylate and etcetera.

The only effect of the aforementioned substances is to fill contour deformities; however, they all have their own side effects.^[1] Having fillers, which can contribute to the physiological structure, has always been a goal for plastic surgeons, thus, autogenous fat transferring has become an appropriate method for this purpose. Among all body tissues, several types of fat have recently been utilized in the forms of fat injection, free fat and dermis fat.^[2]

Literatures related to free fat transferring, which is highly absorptive, date back to 1800 and 1893.^[3] In 1972, dermis fat, which has less absorption and more survival, was transferred clinically to treat head and face atrophy in Romberg patients.^[3]

Generally, there are two theories that explain post-injection fat absorption. The first one is Host Replacement Theory, where the injected fat cells are replaced with the host histocytes; the second, Cell

Survival Theory, emphasizes that fat cells survive due to revascularization and are not digested by histiocytes. Although, the fat volume survival is not exactly predictable, it would be more durable in the form of dermis-fat. According to several studies, up to 40% of fat would be reabsorbed in one year post-operation, therefore, 40% overcorrection is needed to compensate for the fat loss.^[4,5]

There are only a few documented studies available to demonstrate the circumstances under which these types of fat graft have a higher survival rate.

Therefore, the present animal study was conducted to compare different types of strip and punch dermis fat, dermal-fascia-fat and simple fat on the basis of weight maintenance, histomorphologic modifications, angiogenesis process and survival of adipose area, and to investigate whether the angiogenesis improves in the presence of fascia or not.

METHODS

This descriptive animal model study has been conducted with the approval of Isfahan Dentistry Research Center in 2008-2009. This study is the first study that evaluates grafts with weight.

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The excluding criteria was limited to the unpredictable events on dogs and any excluded dog was substituted with a similar one. Statistically, two months of a dog's life is equivalent to 6 months of a human's, so all grafted tissues were analyzed two months post graft operation.

Cases

Twelve female dogs with a similar race, size and age were enrolled into the study and were fed with a standard diet of a farm, one month prior to the operation. All dogs were in a healthy condition according to laboratory and clinical Indices. Surgery was conducted under general anesthesia in two days. To identify, each dog was marked by a microchip.

All dogs were randomly divided into two groups. The first group included 6 dogs for comparison of strip and punch fat grafts. The primary recipient areas were right and left areas of the face (Nasolabial). We put strip fat graft in the right side. In the left side, the strip fat graft was first divided to five punch grafts and then put in the recipient areas. In order to prevent the effect of recipient area fat on the final graft's weight and our measurements, we completely excised the recipient area fat at the time of grafts inset and also to determine the borders we used six-0 Nylone suture around the grafted area. Donor sites were in the trunk near the breasts. All grafts were weighed before inset with a Jewelry scale and for each dog we sent the samples of grafts to pathologists for macroscopic and microscopic examination.

The second group included six dogs. We took fat from same donor area and prepared the samples before the operation as simple fat, dermofat and dermo-fat with fascia. Recipient areas were at right and left of the forehead and all samples were traced externally and internally with six-0 nylon in borders as the same in group one.

Doges were carefully fed for two months in an appropriate farm under the supervision of a veterinarian. After two months, operation was performed under general anesthesia and all grafts were removed precisely. The removed grafts were first weighed with jewelry scale and then stained and evaluated microscopically and macroscopically by an expert pathologist.

Statistical analysis

Data was analyzed by SPSS version 18 (SPSS Inc. , Chicago, IL, USA) using paired t-test.

RESULTS

The mean fat graft weight used in the five different methods is shown in table 1. In order to evaluate the post graft macroscopic modifications, fat grafts were removed after 2 months. Mean \pm SD weight of punch grafts before the operation was 19.6 ± 3.4 gr, while it was 11.4 ± 2.9 gr, two months post-operation, which was meaningful according to paired t-test ($p < 0.001$). On the other hand, in strip fat grafts, the mean weight before operation was 20 ± 3.3 gr and it was 12.1 ± 2.6 gr after the removal of the grafts. The mean weight difference was statistically significant ($p < 0.001$).

According to paired t-test results, the comparison of post operation weight loss between striped and punched grafts was not characteristic ($p = 0.59$).

In circular free fat grafts, the mean weight declined $55.8 \pm 4.9\%$ post operation, and also $43 \pm 4.2\%$ in dermo-fat and $35.67 \pm 4\%$ in dermofat with fascia, respectively, which were meaningful in both groups. Furthermore, the dermal-fascia-fat had the most potential to increase fat graft weight survival (Table 1). Moreover, according to one way ANOVA test, the mean \pm SD of fat loss in 5 groups is statistically significant ($p < 0.001$). The mean difference of fat loss is shown in figure 1.

Grafted specimens were stained with different methods and were analyzed microscopically pre and post operation. In microscopical view of stripe dermis-fat, generally, fat tissue decreased and the remaining fat was surrounded with connective tissue. In 5 out of 6 specimens with reticulin staining, 60% connective tissue and 40% fat tissue with vascularization were observed, in addition, 2 specimens obviously had central collagen synthesis.

Considering microscopic evaluation of punch dermis-fat, fat tissue was highly surrounded with more connective tissue and a vascularized area, even up to 82-90% in some cases; while in one specimen there was an equal area of both connective and fat tissues.

The most significant angiogenesis and collagen synthesis modifications were observed in microscopic view of dermal fascia-fat graft, which were obviously higher than those of fat graft without fascia. Furthermore, fascia dermal-fat specimen showed more post operation angiogenesis, collagen synthesis and fat condensation in comparison with simple dermis-fat and free fat (pictures).

Table 1. Mean ± SD and mean difference of fat graft's weight before and 2 months post OP

Mean ± SD of Wight of graft(gr) Kind of fat graft	Before operation	Two months post graft	Mean difference
Strip graft	20 ± 3.3	12.1 ± 2.6	40 ± 4.4
Punch	19.6 ± 3.4	11.4 ± 2.9	41 ± 5
Free fat no dermis, non-fascia	13.5 ± 1.4	5.9 ± 0.9	55.83 ± 4.2
Dermofat	15.1 ± 2.8	8.6 ± 0.18	43 ± 4.2
Dermofat with fascia	25.7 ± 3	16.4 ± 2.4	35.67 ± 4
P-value	0.88	< 0.001	< 0.001

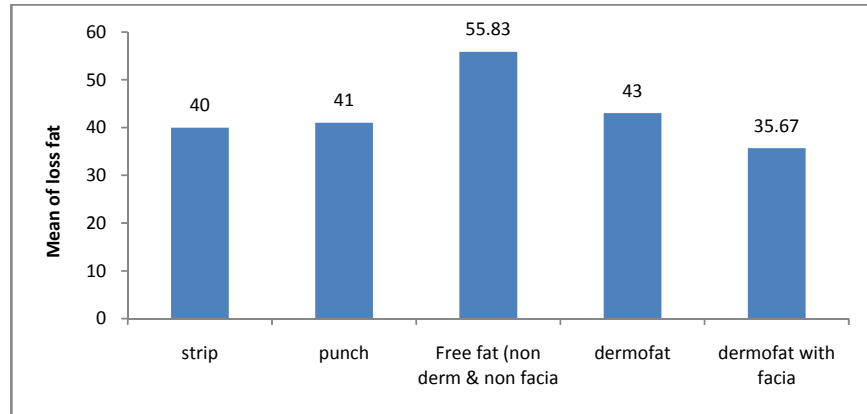
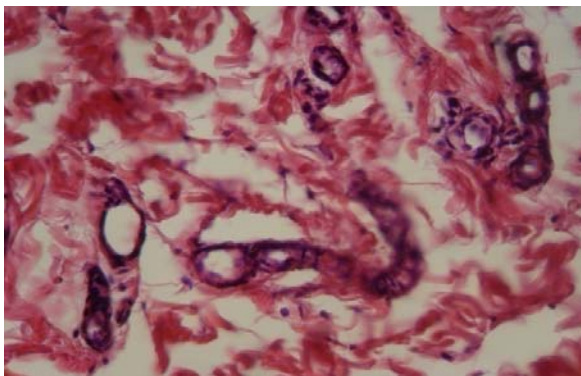
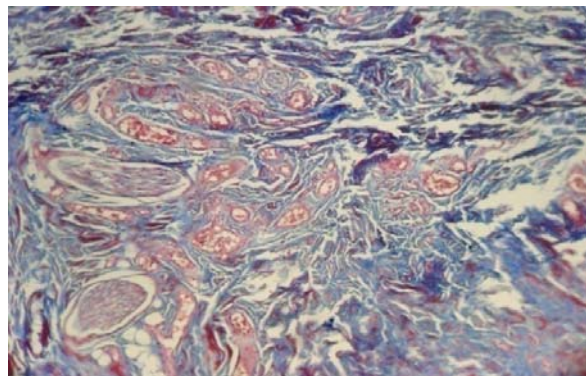


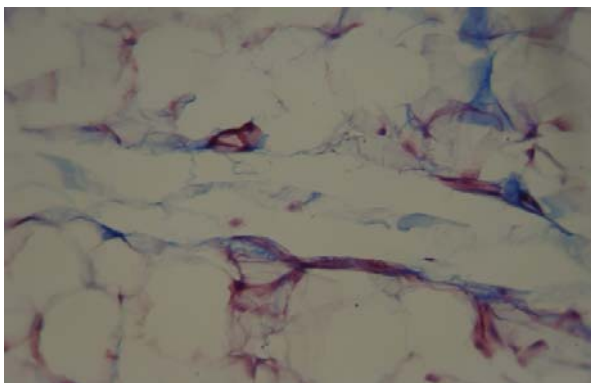
Figure 1. the mean difference of fat loss in the five groups



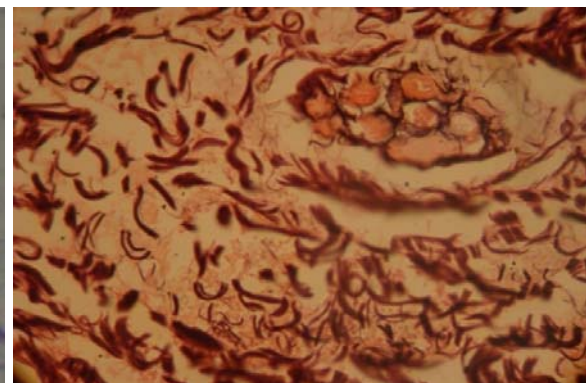
A. Dermal fascia-fat photomicrograph: enhanced angiogenesis (H&E staining, 200×)



B. Dermal fascia-fat photomicrograph: enhanced collagen fibers (Mason Trichrome staining, 200×)



C. Dermis-fat photomicrograph: less increase in collagen fibers (Mason Trichrome staining, 200×)



D. Dermal fascia-fat photomicrograph: enhanced reticulin fibers (Gomory staining, 200×)

DISCUSSION

Autogenous fat grafting was performed in humans as early as 1800. Neuber applied fat graft for the first time to restore pre-orbital defects in 1893.^[6] Simple fat graft was utilized in order to treat hemi-facial atrophy around the early 20th century.^[7] Free dermis-fat graft has also been used to restore facial-defects since 1900. Leaf and Zarem implanted dermis-fat for facial contour defects in 1972.^[8]

Today, dermofat has many indications. It is usually used for nipple areola complex reconstruction, linear facial scleroderma, Romberg,^[9] in orbital reconstruction and in all contour corrections after surgery, for example after parotidectomy, for correction of nasolabial folds in esthetic surgery,^[9-13] and in treatment of lower eyelid malposition as a novel posterior lamellar graft.^[14]

Fat graft has the positive features of an autonomous graft. One simple and routine method of using fat graft is in fat injection. One of the major drawbacks of fat injection is its variable degree of maintenance with variable resorption ranging from 10% to 100%. No clear evidences are available to determine the vascularization event in fat injection, but we know that in dermal fat graft 40-60% of transplanted graft survives with proven vascularization and collagen production.^[15] In our study while we used dermal fat with fascia it caused enhanced revascularization.

Applying dermis-fat grafts have gained more popularity over time to an extent that it is now considered as a common method. According to the studies by Peer, 45% of dermis-fat is absorbed after one year.^[4,5]

Considering available theories, although fat absorption is inevitable, the more revascularization and angiogenesis of grafted area, the better graft volume retention and survival will be.^[16,17]

Since only a few comprehensive studies on fat grafts are available, this study was conducted to investigate microscopic and macroscopic modifications of fat grafts. All Specimens were stained to be evaluated microscopically by pathologists. Trichrome staining methods such as Masson, Van Gibson and Mallory stain extracellular matrix are preferred to other routine H and E staining methods as they more specifically demonstrate collagen elements.^[18,19] The Masson Trichrome method, which characterizes collagen fibers in High Light blue, was used in this study.^[11] Several

reticulin staining methods, which are silver based methods, are available such as Gomerie, Eordemal and Sweet. In addition to collagen fibers, we have used the Gomerie method to stain reticulin fibers as the other extracellular component.^[20,21]

In General, while angiogenesis and collagen synthesis were significantly enhanced in reticulin-stained dermal fascia-fat specimens, we found the least amount of angiogenesis and collagen synthesis in our free fat grafts samples. As seen in figure 2 (Microscopic study), fat tissue was surrounded by connective tissue in both strip and punch dermis-fat graft. In punch graft, increase in collagen synthesis and angiogenesis was observed using reticulin and Masson staining methods, respectively.

Macroscopic studies on post operation grafts showed that that free fat grafts have the most significant weight loss of 55.83% when fascia dermis fat grafts only have 35.67%. Although, there was a decline in punch and strip dermis fat grafts of $41 \pm 5\%$ and $40 \pm 4.4\%$, respectively, the difference between the two methods was not significant ($p = 0.59$).

CONCLUSIONS

According to this study and other mentioned studies in the review of literatures, it seems that dermis-fat graft has an acceptable survival rate. Strip fat graft and punch dermis fat graft had an approximately similar survival rate. On the other hand, collagen synthesis and angiogenesis in fascia dermis-fat is significant and needs to be confirmed in further investigations. In our study we considered the weight and pathological changes of fat grafts but it is recommended to measure the volume indices alongside the pathology and weight in future studies.

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