Lipofilling of the Lips

Ultrastructural Evaluation by Transmission Electron Microscopy of Injected Adipose Tissue

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Objective: To determine if facial autologous fat grafts preserve the morphologic features of adipocytes and guarantee a successful long-term outcome.

Methods: In a previous study, we performed lipofilling in 99 patients between January 1, 1999, and December 31, 2001. In all patients, we performed 3 fat injections at 28-day intervals. We injected 0.4 mL of adipose tissue in the parafrenal area of the upper lip for each treatment. After 4 months, we obtained a biopsy specimen from the same area. We performed an ultrastructural evaluation on freshly harvested fat at the time of harvesting, on stored fat (−30°C) at 8-week and 12-month intervals, and on the biopsy specimens obtained 4 months after treatment.

Results: We observed good preservation of the ultrastructure in the harvested tissue. On histologic examination of the parafrenal area 4 months after grafting, some zones of the biopsy specimens showed putative adipocytes, fat cysts, and collagen fibers adequate for volume increase of the treated area. Ultrastructural images showed lipid droplets intermingled in the connective tissue, phagocytes with internal lipid droplets, and well-preserved adipocytes.

Conclusions: This study demonstrates that by using a less traumatic surgical technique, it is possible to increase the cell survival rate of transplanted fat, thereby maintaining a certain number of viable cells and creating a volume increase in the grafted area. The multiple-stage injection technique seems to be a good method, especially when performed with fat stored at −30°C.

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The Surgical correction of soft tissue defects represents a challenge for plastic, maxillofacial, dermatologic, and otolaryngologic surgeons. All of these specialists are involved in the treatment of orofacial pathologic conditions or in the correction of aesthetic problems. During the past 20 years, many surgical solutions have been used to correct soft tissue defects, including alloplastic and allogenic grafts and other surgical interventions. However, none of these procedures have been free from complications, especially procedures that use alloplastic materials, which can produce unpredictable results.

One surgical option is lipofilling. Fat grafting has been used since the 1800s, but it has been abandoned because of unpredictable cell survival rates. Currently, the development of new liposuction and tissue storage techniques together with many experimental and clinical attempts to increase cell survival rates has prompted a renewed interest in autologous free fat grafting.

The present study investigates the ultrastructural characteristics of autologous fat grafted in the facial soft tissue of 99 patients: 82 (82%) of the patients were treated for lip augmentation, 8 (8%) for facial asymmetries due to cancer, and 9 (9%) for facial asymmetries due to trauma. The main objective of our study is to evaluate which of the 2 mechanisms believed to produce the clinical effect of lipofilling is predominant: cell survival rate or host replacement. In cell survival rate, the grafted tissue is revascularized and preadipocytes therein differentiate into adipocytes, possibly using locally released fat by grafted adipocytes. In host replacement, the grafted adipose tissue degenerates, thereby inducing local inflammatory reaction associated with recruitment of fibroblasts and macrophages, which are associated with phagocytosis. We also analyzed the effects of lipofilling on freshly harvested fat tissue, stored (frozen-thawed) fat, and injected fat.
METHODS

From January 1, 1999, to December 31, 2001, 99 patients were treated with lipofilling procedures in the Department of Maxillo-Facial Surgery at the University of Verona. Patients had been previously operated on for facial pathologic conditions such as cancer resection, trauma, and facial dysmorphisms. Patients also underwent lipofilling for aesthetic reasons. Eighty-two patients received fat grafts in the lips, 9 in the nasolabial area, and 8 in the cheek area. All patients were treated with lipofilling according to a modified form of the technique used by Fournier,11 which is characterized by microlipoinjections.5 Our procedure requires 3 sessions performed at 28-day intervals. All patients signed an informed consent form that described the procedures.

After preparing the medial area of the knee with iodine solution and local anesthesia (2% lidocaine and epinephrine), we injected 70 mL of Klein12 solution and 70 mL of isotonic sodium chloride solution. Fat tissue was harvested manually from this area during the first injection by means of a 3-mm cannula (Medicon Instrumente, Tuttlingen, Germany) and a 20-mL luer lock syringe. The syringe had 2 mL of the physiologic solution inside to prevent trauma on the harvested fat tissue. After the harvesting, fat tissue was immediately washed with the physiologic solution (1:6 ratio in a 30-mL luer lock syringe with anaerobic transfer by means of a 2-mm cannula). Then the fat tissue was transferred to 5-mL luer lock syringes and centrifuged at 3500 rpm for 1 minute.

For those who received grafts on the lateral border of the upper lip, 1.5 mL of fat tissue was injected bilaterally means of a 16-gauge needle inserted vertically to the medial aspect of the upper lip, moving the needle backward while injecting. For those who received grafts in the nasolabial area, we inserted a 16-gauge needle into the inferior portion of the nasolabial sulcus, injecting 2.9 mL of fat with a backward direction, with the patient under local anesthesia. Finally, those patients who underwent a mandibular resection for benign oral lesions were placed under general anesthesia and received 30 mL of fat by inserting the needle behind the retroauricular area to avoid facial nerve damage. All graft materials were injected subcutaneously and intramuscularly. The remaining fat tissue was frozen at −30°C and then used for the second and third injections.

We performed 3 injections for overcorrection (every 28 days) because of partial reabsorption of the graft. The 28-day injection intervals were chosen to avoid further trauma on the grafted fat that was going to be revascularized between days 4 and 2813,14 and to allow the local inflammatory response to decrease. Three injections are necessary to reach adequate fat volume, since we observed a reabsorption rate of approximately 64%, 8 to 10 days posttreatment. Partial reabsorption proved to be advantageous because it allowed refinement of the final result by means of clinical controls and pictures. Ten patients received an injection of 0.4 mL of fat in the right paranasal area of the upper lip at each treatment (freshly harvested fat for the first injection and frozen-thawed fat for the second and third). A biopsy specimen was obtained from the same site 4 months after the first injection and was processed for transmission electron microscopy. The patients had no complications, such as hematoma or pain, in the harvested and grafted areas. Additional injection outcomes will be investigated in clinical follow-up to obtain a precise measure of the residual fat graft and of the newly formed volume.

MORPHOLOGIC FINDINGS

Specimens of freshly harvested fat were obtained, fat was stored at −30°C, and biopsy specimens were obtained from the graft site 4 months after the first injection. For transmission electron microscopy, specimens were fixed in 2% glutaraldehyde in 0.1M phosphate buffer for 2 to 3 hours at 4°C, postfixed in aqueous osmium tetroxide (1% vol/vol), washed, dehydrated through graded acetones, and embedded in a mixture of epoxy resins (Epon and Araldite; SPI-Chem, Westchester, Pa). Two-micrometer-thick sections were obtained from plastic blocks, placed on glass slides, stained with toluidine blue dye, and viewed and photographed in a photomicroscope (Leitz Orthoplan; Leica Microsystems AG, Wetzlar, Germany). Ultra-thin sections were obtained on an ultramicrotome (Ultra-Cut; Reichert Jun, Vienna, Austria), collected on copper grids, stained with lead citrate, and observed in a transmission electron microscope (Zeiss EM10; Carl Zeiss, Oberkochen, Germany) operated at 60 kV.

CLINICAL CASES

Patient 1

We treated a 31-year-old woman who had previously undergone procedures for correction of prognathism and hypoplasia of the upper maxilla. She disliked her thin upper lip (Figure 1). Lipofilling of both the upper and inferior lips was performed by procedures described herein. We performed 3 injections at 28-day intervals. After each injection, the lips were slightly edematous, but edema completely disappeared 8 to 10 days posttreatment. Photographs were taken 3 weeks after each injection and showed better definition of the lips, a better outline of the Cupid’s bow, and a good final aesthetic result (Figure 2).

Patient 2

A 23-year-old woman treated for prognathism and hypoplasia of the upper maxilla several years ago underwent surgery to correct nasal septal deviation. Before surgery, the patient asked about getting the volume of the zygomatic area and the lips augmented (Figure 3). Lipofilling was performed as described herein. The photographs taken 8 months postinjection showed good increase of volume in all the treated areas (Figure 4).

RESULTS

Morphologic examination of fat tissue harvested from the medial area of the knee regularly showed the presence of typical white monovacuolar adipocytes. Light microscopy findings (Figure 5A) showed a modest widening (probably edema) of the intercellular space, probably due to the harvesting procedure. Ultrastructural examination of adipocytes (Figure 6A) revealed good preservation of the ultrastructure in typical white fat cells: the nucleus was displaced at the periphery of the cell by a large adipose vacuole devoid of membrane, and the scanty perinucleus cytoplasm contained a few organelles, such as small mitochondria and short profiles of endoplasmic reticulum.

Histologic examination findings of stored fat samples (Figure 5B) showed that the general architecture of the tissue was maintained despite obvious reduction of the intercellular connective tissue. On transmission electron microscopic examination, fat cells showed several morphologic alterations, such as interruptions of the plasma membrane, partial loss of the lipid deposit, mitochondria lesions, and loss of the basal lamina.

Figure 4

A) showed a modest widening

Figure 5

B) showed that the general architecture of

Figure 6

A) revealed good preservation

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Histologic examination findings from the biopsy specimens revealed a spectrum of morphologic aspects. In some areas, connective tissue was rather dense and organized; nevertheless, putative adipocytes and small lipid droplets of different sizes were present therein (Figure 5C). Elsewhere, putative adipocytes and lipid droplets were more abundant, and reactive cells (sometimes embracing putative adipocytes, Figure 5E) and newly formed blood vessels were prominent (Figure 5D and E). At the ultrastructural level, biopsy tissue samples contained abundant collagen fibers of a normal period (350-600 µm, the same as for normal collagen fibers), often in bundles of different directions (Figure 6B). Lipid droplets of markedly different sizes, sometimes associated with cytoplasmic debris, were intermingled in the connective tissue. In the surrounding areas, cells that contained small, membrane-bound lipid droplets and showed the ultrastructural features of phagocytes were found (Figure 6C). Similar cells were also found in prenecrotic form (Figure 6D). At light microscopic and transmission electron microscopic levels (Figure 6E), well-preserved adipocytes were found. These cells showed a peripheral basal
lamina, abundant micropinocytotic vesicles at the plasma membrane, regular intracellular organs, and a multivacular lipid deposit with a larger central drop and many smaller peripheral droplets.

We observed no pain or complications such as hematoma in the harvest and graft sites in any of the patients. The medial part of the knee was harmonic, and the lip and skin texture after 4 months was good. No sensitivity damage to the lips was present. All patients were happy with the results of the procedures. They experienced no complications and agreed to be treated again if necessary.

Figure 3. A, Frontal view of patient 2 before treatment. B, Lips of patient 2 before treatment.

Figure 4. A, Frontal view of patient 2, 4 months after treatment. B, Lips of patient 2, 4 months after treatment.

FRESHLY HARVESTED ADIPOSE TISSUE

Adipose tissue fragments freshly harvested for lipofilling showed generally well-preserved morphologic features of adipocytes in all cases, at both the light microscopic and transmission electron microscopic levels. In most cells, the cell membrane and key cytoplasmic or-
ganelles, such as mitochondria, showed regular ultrastructure. Therefore, despite the trauma from harvesting tissue and the preparative procedures for injection, it is apparent that the smaller the diameter of the adipocyte (<1000 µm), the higher the percentage of viable adipocytes at the time of injection, especially if a 3-mm cannula is used.

FROZEN-THAWED ADIPOSE TISSUE

Transmission electron microscopy of frozen-thawed tissue showed a few relatively well-preserved adipocytes and numerous cells with obvious morphologic changes. Such changes are attributable to the well-known disruption of the proteolipid membrane complex associated with the freezing-thawing cycle. These results suggest that thawed fat tissue injected during lipofilling contains a mixture of lipid droplets derived from adipocyte lysis and a small number of preserved cells. The number of injected viable cells would be very few according to the animal study by Lidagoster et al.15

PARAFRENAL AREA TISSUE

Morphologic examination findings of the biopsy specimens obtained 4 months' posttreatment were different yet similar to those of a previous study by our group.5 The tissue of the graft site showed proper cells and cellular fragments as a result of a partial necrosis of the injected fat and local reaction to the grafted material located in the same area, perhaps creating fat cysts. In all patients, variable amounts of connective tissue, fat droplets, fat cells, phagocytes, and newly formed vessels were found in the graft material as similarly described by Coleman et al.16 The presence of newly formed vessels indicates that the injected fat is to some extent revascularized, as previously demonstrated in animal models.17 Extravascular red blood cells found in our material are attributable to hemorrhage at the time of biopsy.

Regular morphologic adipocytes in the graft site were found and presented structural evidence of metabolic activity (membrane micropinocytosis). These cells were isolated or in small groups, often surrounded by connective tissue. Fat droplets were often sparse in the connective tissue, which differed from adipocytes because of the absence of cell membrane and the peripheral cytoplasm rim. Fat droplets in connective tissue were different sizes (1-2 µm to tens of micrometers) and were isolated or in groups. Probably, these are remnants of adipocytes injected during lipofilling. Phagocytes, either isolated or in groups, were found in areas where the reactive connective tissue is less organized. These cells contained numerous lipid droplets surrounded by cell membrane and cytoplasmic granules. These cells, surviving in the graft site 4 months after grafting, seem to be able to sequester a significant amount of fat, increasing the tissue volume. The presence of morphologically preserved adipocytes in tissue biopsy specimens suggests that a limited amount of injected fat cells survives for a long period in the graft site.

No clear evidence of differentiating adipose cells (preadipocytes) was found in studies by Har-Shai et al 6 and Yuksel et al.9,10 Therefore, it seems probable that, un-
Under our experimental conditions, this lipofilling procedure does not induce any significant de novo adipogenesis. This article will be followed by a study on the third clinical experience of our group. In particular, we believe that although our technique will probably not increase the cell survival rate, it will demonstrate that there...
is ultrastructural evidence of collagen, putative adipocyte, and fat cyst volume increases. These outcomes are well controlled with the 3-stage injection technique. The 28-day injection interval stimulates collagen storage with the inflammatory response, allows revascularization of the grafted fat, and gives the surgeon accurate control of the desired final volume. Nevertheless, 4 months after the first injection, a significant amount of fat persists in the graft site as free droplets and intracellular droplets are accompanied by a light connective reaction. The purpose of our research is to possibly induce differentiation of mesenchymal cells into preadipocytes and adipocytes by means of stimulating factors and to establish an easy volume measurement technique.

**CONCLUSIONS**

Fat tissue transplantation represents a good alternative to traditional techniques in the correction of facial soft tissue defects because of low cell morbidity and easy execution. Several authors have studied fat tissue transplantation, providing scientific contributions to improve the viability and longevity of the correction made by lipofilling. The purpose of our study was to analyze the cell survival rate of the injected fat by ultrastructural analysis. This study seems to confirm that by using a less traumatic surgical technique, it is possible to increase the cell survival rate of transplanted fat. Furthermore, the 3-stage injection technique seems to be a good method, especially when fat is stored at −30°C. Future studies will aim to increase proliferation of local and/or grafted preadipocytes, to inject fat tissue with specific adipocyte proliferation–associated factors, and to improve injection technique and volume measurement.

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**REFERENCES**


