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ORIGINAL ARTICLE

The Effect of Epidermal Growth Factor on Autogenous Fat Graft

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Abstract Autogenous fat grafting is widely used for the correction of soft tissue contour deformity. However, the high absorption rate results in the need for overcorrection, and graft longevity is unpredictable. The authors hypothesized that epidermal growth factor (EGF), a potent stimulator of neovascularization, would improve fat graft survival. The experiment used two groups of New Zealand White Rabbit ear. Inguinal fat was harvested and injected with EGF or saline ($n = 24$, each group). The 48 cases of fat grafting were managed for observation of volume and morphologic change. The fat was harvested 3 months after the autogenous graft. The survival rate and the degree of neovascularization were measured. The grafts in the EGF group had a significantly higher survival rate than those in the control group. Histologic examination of the grafts demonstrated an increase in neovascularization and maintenance of fat cell morphology. These findings show that EGF can enhance fat graft survival and degree of neovascularization. Further well-controlled studies are required before EGF is used for clinical purposes.

Keywords Autogenous fat graft · Epidermal growth factor · Soft tissue contour deformity

Autogenous fat grafting is a method used to fill soft tissue or reduce wrinkles. It is widely used for reconstruction and cosmetic surgery because it results in fewer scars and a low risk of complications such as foreign body reaction or infection.

Autogenous fat grafting was first introduced in 1893 by Neuber [1] for unilateral facial atrophy, Romberg's disease, atrophic scar, and breast operations as a mass graft. In 1911, Brunning discovered a new method involving insertion of roughly ground fat tissue into a recipient's subcutaneous layer for soft tissue filling [2].

Since the 1980s, liposuction has been commonly performed in a variety of studies for fat tissue harvest. Illouz [3] and Founier [4] invented a new method to harvest and graft fat tissue by syringe, named the "microfat graft" [5]. Autogenous fat grafting has improved over time, but problems still exist, such as the slow reabsorption of 20% to 50% of fat tissue 3 to 6 months after treatment. Moreover, the tissue survival rate is not consistent [6, 7]. Therefore, many studies have tried to increase the fat tissue survival rate. For example, efforts have been made to increase durable fat cell numbers by fat tissue concentration, to remove inflammatory mediators by washing, and to minimize mechanical damage to fat tissue by emitting negative pressure or overcharging during fat tissue harvesting [8]. To improve the grafted fat survival rate, new methods such as adding insulin, vitamin E, fibrin glue, or fibroblast growth factor (FGF) and performing platelet-rich plasma have achieved satisfactory results.

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The mechanism of fat tissue survival and absorption is not fully understood. Fat tissue survives by nutrient diffusion from the serum within the first 48 h. After this, fat tissue starts to regenerate through the vascular system [9]. Absorbed fat tissue is removed by macrophages and replaced with fibrous or cystic tissue [9].

Neovascularization in the early period after fat tissue grafting is known to be important in fat tissue survival. Studies concerning growth factors and wound-healing materials that help neovascularization are ongoing [10]. Epidermal growth factor (EGF) stimulates neovascularization in various types of wounds [11]. Also, recombinant human EGF (rhEGF) mass produced for use in chronic wounds is recognized as effective for this purpose.

In a previous study, Viterbo et al. [12] showed that rabbit ear was the best site for studies of fat integration and that fat block grafts showed better survival rates than lipoaspirated fat. The study presented in this report used the New Zealand White Rabbit to compare the grafted fat tissue survival rate of EGF-treated and control groups by comparing fat tissue survival rates and neovascularization.

Materials and Methods

Animals and Medication

In this study, 12 New Zealand White Rabbits received autogenous fat grafts. Fat tissue then was retrieved after treatment for comparison of tissue survival and degree of neovascularization. The pretreatment weight was 2 to 2.5 kg (mean, 2.3 kg), and the mean weight gain during the study was 410 g. Every step in the animal study was performed according to the guidelines of the Ewha Woman's University Medical School (Seoul, Korea). The EGF was obtained from the biotechnology department of Dawoong Pharmaceutical Company (Seoul, Korea).

It was aseptically brought to a volume of 5 ml by mixing 0.005% powder with normal saline. The EGF then was injected into grafted fat tissue. Both ears of 12 rabbits were used in this study (24 ears total), and each ear was divided into two areas for fat grafting (48 groups total). The right ears injected with normal saline comprised the control group. The left ears injected with EGF solution comprised the experimental group.

Fat Graft

The rabbits were anesthetized by an intramuscular injection of a mixture of Zoletile (Tilemine 125 mg, Zolazepam 125 mg; Vibrac®, NH, USA) 15 mg/kg and Rompun (xylazine hydrochloride, 23.32 mg/ml; Bayer Korea Corp., Seoul, Korea) 5 mg/kg. For the donor site, the right inguinal area was shaved and cleaned using betadine. The inguinal fat pad then was exposed by a 3-cm incision. Pure fat was harvested using a minimized procedure to reduce fat tissue damage. Harvested fat tissue was cleared with normal saline, dried with gauze, and divided into four parts with a total mass of 0.5 ml (Fig. 1). The volume of fat was measured using a 2-ml syringe filled with 1 ml of normal saline. Each fat group was measured with a combined mass to minimize the volume error range.

The area of the rabbit's ear in which fat tissue does not naturally exist was divided in half. A 0.5-cm incision was made in four areas of each rabbit. The fat was grafted between the skin and the perichondrium. The incision site was sutured, and the right ear was injected with 0.5 ml of normal saline using a 26-gauge needle. The left ear was injected with 0.5 ml of EGF (Fig. 2). Until postoperative day 1, aseptic dressings were used, and an open dressing was continued until sutures were removed on postoperative day 10. A 0.5-ml volume of EGF and normal saline were reinjected on postoperative day 1, then 1 week, 2 weeks, and 3 weeks after surgery for a total of four treatments. Fat was grafted as a round mass to reduce error.

Fat Tissue Harvesting

During the 3 months after graft placement and after the gross volume change had been checked, fat tissue was harvested. An incision was performed 1 cm apart from the graft site to expose the grafted fat tissue. The skin around the grafted fat tissue also was harvested attached to the fat tissue.

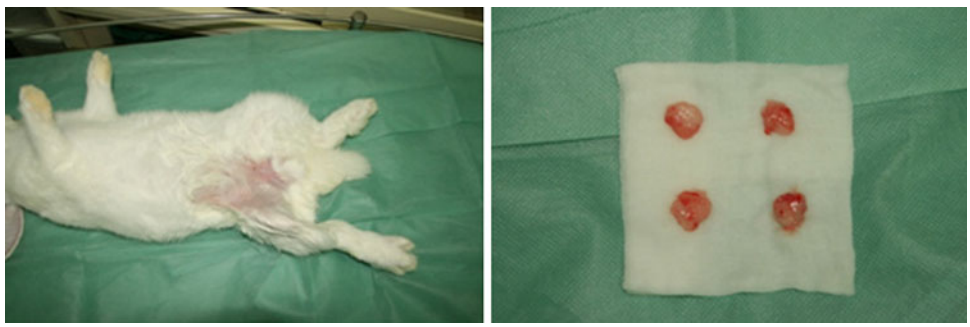

Fig. 1 Fat donor site and grafted fat tissue

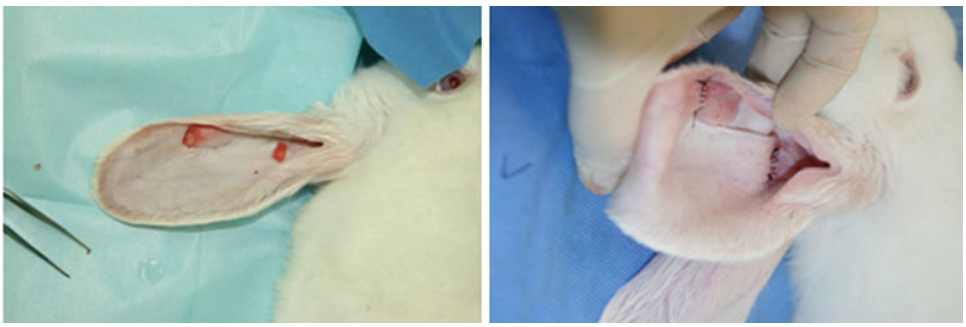

Fig. 2 Process of grafting fat tissue

Volume Analysis

Harvested fat tissue was cleared with normal saline and dried with gauze. A 2-ml syringe with 1 ml of normal saline was used to measure volume change. The volume of the harvested fat tissue was studied by *t* test (SPSS, v12; SPSS Inc., Chicago, IL, USA), mean, and standard deviation. The fat tissue survival rate was calculated using the change in fat tissue volume.

Histologic Analysis

Tissue samples were fixed in formalin, then stained using hematoxylin-eosin (H&E) and Masson's trichrome. The fat tissue was examined for the morphology of adipocytes and the degree of neovascularization microscopically using ×10 and ×40 objectives. Neovascularization was assessed by measuring the number of capillaries in 20 fields in each group on H&E stained slides (×400 magnification; 0.13 mm^2). Measurements were performed by one blinded pathologist.

Results

At gross examination before harvest, the volume of fat and the tissue appearance were well maintained in the group treated with EGF. However, in the control group, grafted fat differed widely from that in the EGF group (Fig. 3). During the harvesting procedure, the fat tissue in the EGF group was well detached and showed less adhesion than that in the control group (Fig. 4). Particularly when

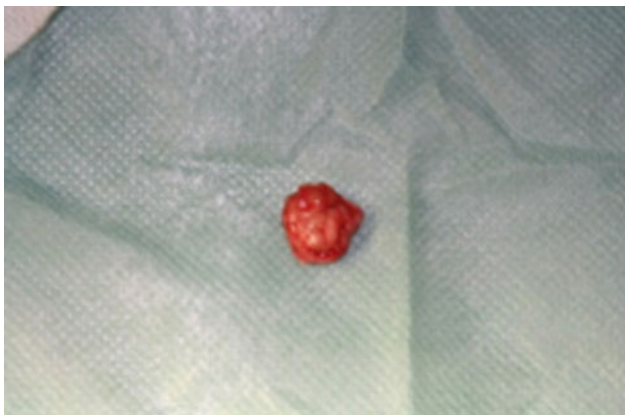

Fig. 4 Fat tissue harvested from the epidermal growth factor (EGF) group

observed grossly after harvesting, the cartilage of the control group had prominent adhesions and fibrosis. The mean volume was 0.35 ml in the EGF group versus 0.28 ml in the control group. The survival rate was 70% and 56%, respectively. This result correlates with the gross observation, and *t* test analysis results demonstrated an improved tissue survival rate in the EGF group ($p = 0.002$) (Fig. 5).

In the control group, fat cells were deformed, with fewer capillaries and a lower density. Adhesion to adjacent tissues and fibrosis also were predominant. The mean number of vessels was 3.89 ± 1.60 per field in the experimental group and 1.78 ± 1.02 vessels per field in control group (Fig. 6). The control group differed significantly from the experimental group ($p < 0.05$). At histologic analysis, the

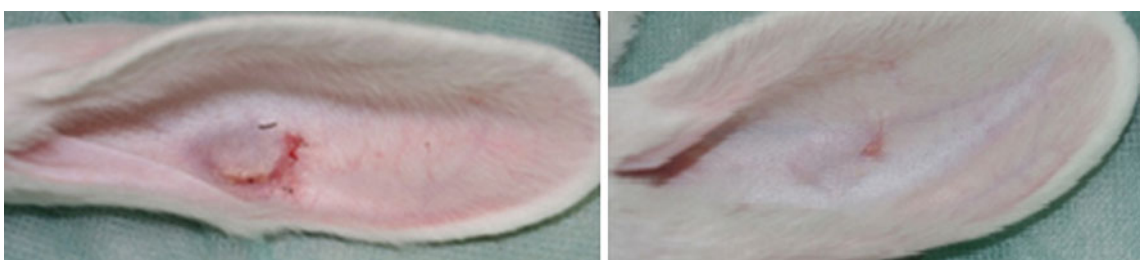

Fig. 3 Gross change in recipient site after grafting (postoperative week 6). *Left* Epidermal growth factor (EGF) group. *Right* Control group

	Mean volume	Survival rate	Standard deviation of volume
Experimental group (EGF group, N=24)	0.35ml	70%	0.037
Control group (Normal saline, N=24)	0.28ml	56%	0.025

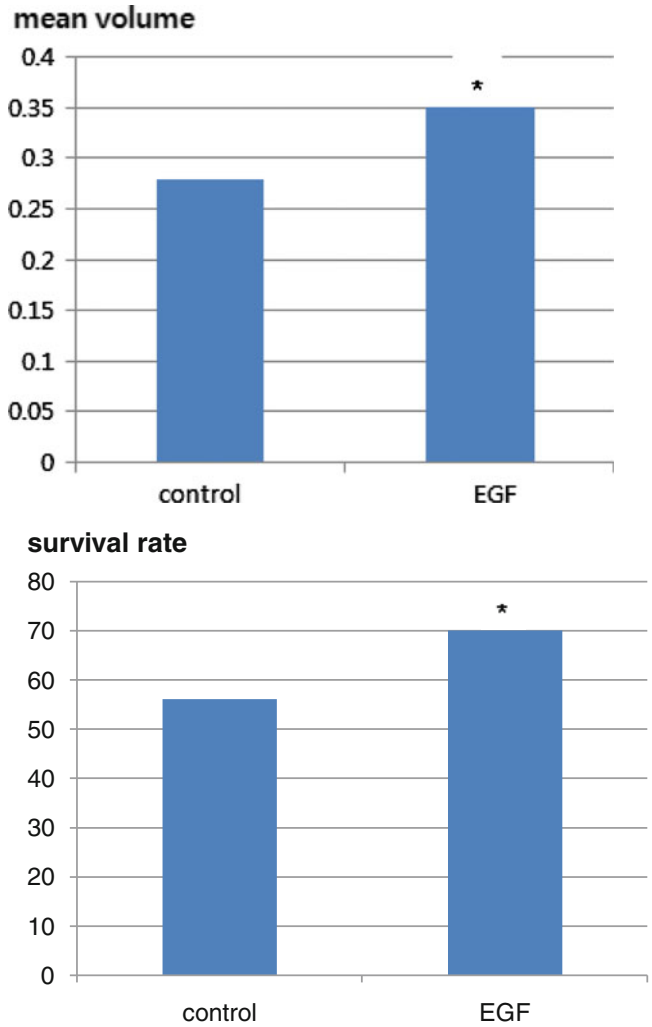

Fig. 5 Volume change in autogenous fat grafts ($p = 0.002$, paired t test)

adipocytes of the EGF group were well maintained, with less necrosis and deformation, and the fat cells were more dense than those of the control group (Figs. 7, 8).

Discussion

Because fat tissue is abundant in the body, simple to harvest, has little immunologic rejection, and is easy to treat, autogenous fat graft is widely used not only for cosmetic surgery but also for reconstruction of facial volume and outline reformation. However, autogenous fat grafting does have a few drawbacks such as a variable tissue survival rate and reabsorption after graft. Therefore, studies to improve the tissue survival rate and maintenance after the graft procedure often are performed.

The tissue survival rate varies among reported studies. In the 1950s, fat tissue was grafted as a mass

	Mean number of capillaries
Control	1.78 ± 1.02
EGF	3.89 ± 1.60

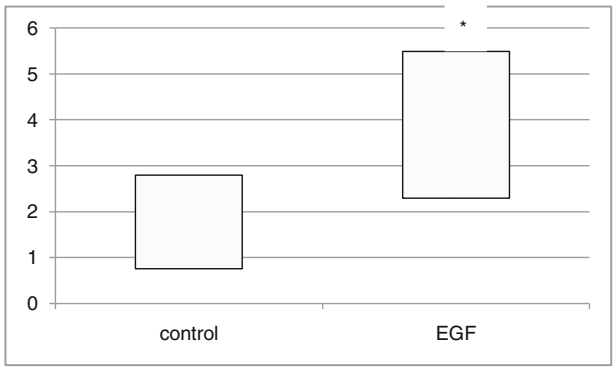

Fig. 6 *Top* Histologic vascular density results. *Bottom* Graphic representation of the histologic vascular density results. The experimental and control groups differed significantly (*) ($p < 0.05$)

transplantation. With this method, the survival rate was a maximum of 50%, as reported by Peer [13], but the average tissue survival rate was much lower. Methods for grafting fat tissue after rough grinding also were introduced. Again, the tissue survival rate was unsatisfactory. In the 1980s, as liposuction became popular, materials aspirated from liposuction were used in autogenous fat grafting.

To improve tissue survival rates, Coleman [14] recommended grafting layers thinner than 1 mm with a syringe. On the other hand, Chajchir et al. [15] recommended a 50% overgraft in anticipation of fat reabsorption. In the 1990s, many studies were performed, but the reported fat survival rate varied from 50% to 80% [8, 16, 17].

In an animal study, Viterbo et al. [12] first introduced the rabbit ear for the study of fat graft integration. The rabbit ear showed lower degrees of inflammation and fewer cystic cavities in fat block grafts. Also, rabbit ear was the best site for studies of fat integration. Subsequently, rabbit ear methods were widely used for studying fat integration.

Survival and reabsorption mechanisms after fat grafting are poorly understood, and further studies are projected. In 1923, Neuhof and Hirshfield announced that grafted fat tissue changes initially into preadipocytes, ultimately becoming a fat tissue called "metaplastic fat." They insisted on a "host cell replacement theory" in which grafted fat cells finally expire, to be replaced by the fibrous tissue of metaplastic fat. After 30 years of consistent study, they discovered that not all the cells died and that about 50% survived in the tissue and maintained volume.

Recent discoveries show that undifferentiated fat cells play a significant role in tissue survival. Preadipocytes or fat stem cells contained in autogenous fat grafts are resistant to damage during harvest, can survive a prolonged period with minimal nutrition, and have the ability to differentiate to more mature cells [14]. To maximize tissue survival during harvest, minimum negative pressure is

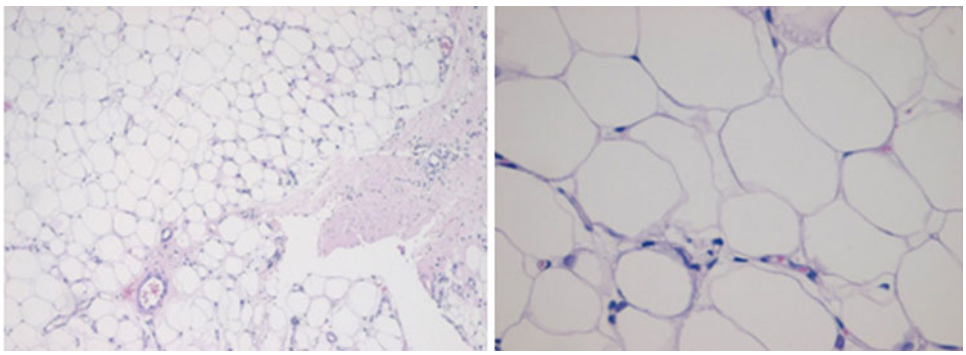

Fig. 7 Histologic finding of epidermal growth factor (EGF). *Left* Hematoxylin and eosin (H&E) stain (magnification ×10). *Right* H&E stain (magnification ×40)

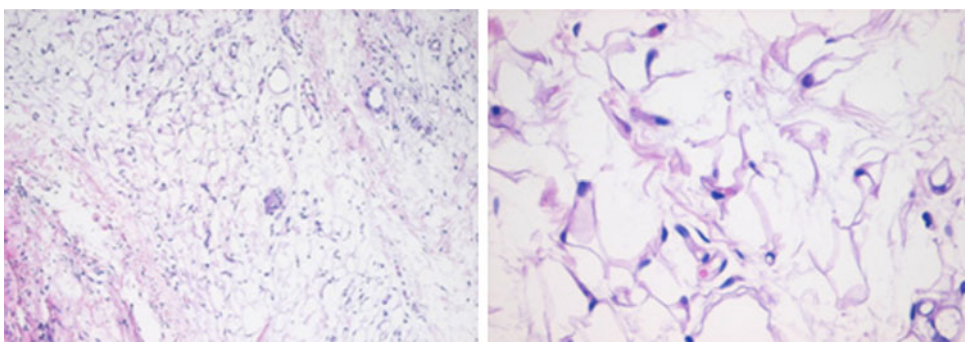

Fig. 8 Histologic finding of the saline group. *Left* Hematoxylin and eosin (H&E) stain (magnification ×10). *Right* H&E stain (magnification ×40)

applied, a special cannula is used with the proper technique, the procedure is simplified, and the depth and placement of the injection are carefully selected. However, no universally accepted standard technique currently exists.

The addition of insulin, vitamin E, fibrin glue, FGF, or platelet concentration plasma during grafting has led to some positive results, with the limitation of a more complicated procedure requiring additional training. The safety of the procedure is not proven. It is widely known that not only preadipocytes and fat stem cells are important but also neovascular formation between days 3 and 21, and studies concerning growth factors and materials that stimulate neovascularization for wound repair are actively ongoing. In particular, platelet-rich plasma currently is being introduced clinically since studies showing that it aids in wound repair and increases fat survival. However, problems still exist regarding the invasiveness of the procedure, the lack of standardized tools, infection risk, and unsatisfying results [10, 16]. One of the well-known growth factors, EGF, stimulates mitosis; activates platelets, macrophages, and fibroblasts; and enhances neovascularization to promote wound healing. It has a synergistic effect with other growth factors [11, 18]. A 0.005% concentration of EGF has been marketed. It includes 53 amino acids and, similar to human EGF, it is recognized as effective in chronic wound treatment.

We considered using EGF in this study due to the importance of primary neovascularization in increasing fat graft survival. Among the growth factors, EGF appears to be one of the most important factors. It is widely used in diabetes mellitus foot and partial epithelial grafts, but it rarely has been studied for use after autogenous fat grafting. We thought rapid termination of wound healing in fat grafts would result in less volume loss and better maintenance of shape.

The fat survival in this study was similar to that reported clinically. The EGF group showed a 70% fat survival rate, which represents statistically significant improvement. Considering that mass grafting has a lower survival rate than fat injection, our results represent a relatively high survival rate. Also, no leukocyte infiltration or cystic cavity formation occurred. Our results were similar to those of Viterbo et al. [12].

The results of our study point to the importance of neovascularization and the role of growth factors. Gross comparison with the control group shows that fat from the EGF group had a stable form because of increased cell survival and reduced inflammation. At histologic analysis, the EGF group had more fat cells with a higher cell density.

When autogenous fat grafting is performed clinically, the way that cells maintain their shape also is significant. Fibrosis and adhesion can cause a disturbance in fat tissue reinjection. Another risk factor that reduces satisfaction is fat tissue that can be palpated as a hard mass under a thin layer of skin or appears similar to scar tissue. From this point of view, EGF can improve patient satisfaction by maintaining the normal architecture of fat tissue.

According to some experimental studies, it is possible that the excessive scar reaction and fibrosis result from excessive collagen deposition at the site of wound healing. At this writing, transforming growth factor β (TGFβ) is known to be the key regulator of excessive contracture. Recently, it was demonstrated that EGF might negatively regulate the role of TGFβ without having any influence on fibrosis or scar formation [19]. For example, topically applied EGF enhances the wound repair process while playing a role in decreasing the formation of excessive scar tissue [20]. In addition, local application of EGF decreases the histamine level in tissue. Because the increased histamine content in a wound area mainly causes abnormal collagen formation and fibrosis, a topical EGF treatment can have a beneficial effect on the wound-healing process without immoderate scar formation and fibrosis. Overall, it is possible that EGF accelerates the natural wound-healing rate while simultaneously suppressing the formation of scar and fibrosis [21].

However, the method of EGF delivery needs further consideration. In 1999, Brown [22] compared tension after injecting EGF into the backs of mice. During the study, they injected EGF into the fascia of the back. Another studiy also used this method [23]. However, further studies are needed to investigate safety, proper frequency, amount, timing, and potential complications.

Conclusion

In this study, grafted fat maintained form, had fewer adhesions, and exhibited a higher tissue survival rate with EGF treatment. At histologic analysis, the EGF group contained a greater number of fat cells with a higher density, and neovascularization was detected. Therefore, the use of EGF can improve the result of autogenous fat grafts. Further studies exploring the precise mechanism of action would be beneficial as well as further investigation into the frequency of use and potential complications with the goal of achieving the optimal clinical results.

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