Objective: To increase the viability of fat grafts using vascular endothelial growth factor (VEGF) in a calcium alginate microsphere controlled release system.

Design: Twenty-four rats were divided into 4 groups of 6 rats each. Group 1 was the preconditioning group in which VEGF was applied prior to the fat grafting. In group 2, VEGF was given at the time of the grafting. In group 3, an empty microsphere was added to the grafting material. The fourth group, which received the fat graft only, was the control group. At the 90th day, samples of the fat grafts were weighed and compared with preimplantation weights.

Results: The graft viability ratios of the first 3 groups were significantly higher than those of the control group. The relative adipocyte index was significantly higher in the first and second groups compared with the control group and group 3. Consistent with the literature, VEGF used both in the preconditioning procedure and simultaneously with the grafting procedure increased the graft viability ratio and relative adipocyte index.

Conclusion: This study suggests that VEGF-induced preconditioning of the recipient bed improves fat graft viability via increased revascularization.


when volume replacement for any part of the body is an issue, autogenous fat is usually considered the first filler material of choice in plastic surgery because the fat is autogenous, biologically compatible, and available in a sufficient amount. However, it has some drawbacks, such as unpredictable necrosis and cyst formation during the postoperative period. Long-term follow-up studies after fat grafting have usually revealed a 20% to 50% volume loss according to the initial amount. Because of this unpredictable volume loss, overcorrection and/or multiple stages are required to meet the patients’ needs. So, the viability and durability of fat grafts are still not definite.

We have been trying to increase the survival of the fat grafts by using various methods (eg, by increasing the number of adipocytes via concentration, rinsing the graft several times to eliminate inflammatory mediators, using a low-pressure nontraumatic technique to spare fragile adipocytes, and using steroids and antioxidants).

It has been shown that onset of graft vascularization is evident by the second day after placement. The survival of adipocytes depends on the vascular supply of the surrounding tissue. This observation implies that a rich vascular supply may decrease graft loss. Depending on this evidence, muscular recipient sites have been studied by some authors. Guerrerosantos et al and Aygit et al injected fat into the muscle and observed that the viability of the graft increased. Baran et al increased the vascularity of the recipient bed by inducing capsular tissue around a silicone sheet prior to application of the fat. Yi et al showed that the VEGF gene transfected with adenovirus vector increased fat graft survival in nude mice. Aiko et al and Hutley et al suggested that some soluble factors originating from endothelial cells promote adipogenesis. Fukumura et al demonstrated that vascular endothelial growth factor (VEGF) might have positive effects on preadipocyte differentiation through VEGF receptor-2 (VEGFR2) in paracrine fashion.

Based on previous studies, and considering recipient bed preparation and graft manipulation, we aimed to assess the ef-
The effect of VEGF on the viability of the fat graft through increased recipient bed vascularity and earlier VEGF supplementation for graft.

**METHODS**

Twenty-four male Wistar rats with a mean (SD) weight of 340 (20) g were taken care of according to the Animal Users and Care Guide of the National Institutes of Health Laboratory. All surgical procedures were performed under sterile conditions.

**PREPARATION OF THE MICROSPHERES**

The microspheres were prepared following the guides as in the literature. A solution of sodium alginate, 2% (Sigma, San Diego, California), in sodium chloride, 0.9%, was prepared. The solution was centrifuged at 20,000 × g for 3 hours to remove insoluble particles. The solution was sterilized using UV light, keeping the tubes overnight.

We used VEGF165 (Sigma) as a dilution in 0.4 mL of sodium chloride, 0.9%. Immediately after preparation, the dilution was added into 3 mL of the alginate solution. After gentle mixing, the solution was extruded through a 23-gauge hypodermic needle into a glass beaker containing 0.1M of calcium chloride (Sigma-Aldrich, San Diego, California) solution. The insolubilization reaction of alginate and calcium resulted with encapsulation of VEGF165 in calcium alginate microspheres. The procedure was performed in a cooled room (4°C), and the mixture was the stirred with magnetic stirring device overnight. Control group microspheres were prepared with sodium chloride, 0.9%, only (Figure 1 and Figure 2A).

**GROUPS**

The 24 rats were divided into 4 groups of 6 rats each. Group 1 was the preconditioning group, in which VEGF was applied prior to the fat grafting. In group 2, VEGF was given at the time of the grafting. In group 3, an empty microsphere was added to the grafting material. The fat graft-only group was the control group (Figure 3).

**SURGERY**

In group 1, microspheres containing 2 µg/0.1 mL VEGF were injected subdermally to the dorsal interscapular region of the rats using a 23-gauge needle 21 days prior to the fat grafting. The injection site was marked with a Prolene suture.

On the day of the operation, all rats were anesthetized with 0.5-mL intraperitoneal injections of the anesthetic mixture (ketamine hydrochloride, 100/mL, and xylazine hydrochloride, 20 mg/mL). An oblique incision was made in either inguinal region, and the skin and panniculus carnosus complex was elevated to expose the inguinal adipofascial flap (Figure 2B). The fat obtained was cut into pieces approximately 2 to 4 mm in length in all dimensions, washed in saline, and blotted dry with sterile, lint-free paper. The prepared fat pieces were combined and weighed to create grafting portions with a mean (SD) weight of 0.5 (0.1) g (Figure 2C and D). Pockets were created in the loose areolar tissue under the panniculus carnosus on the dorsal interscapular region of each rat.

In group 2, the portions of the fat were mixed with calcium alginate microspheres containing 2 µg/0.1 mL VEGF and then were placed into the pockets (Figure 2E). In group 3, unloaded microspheres were added to the grafting material. In group 1 and the control group, the raw fat graft was placed in the pockets. After the placement of the fat grafts, the pockets were closed with 5-0 monofilament nonabsorbable sutures that also served as a marker for the localization and analysis. The donor area incision was sutured with 5-0 nonabsorbable sutures.

**SAMPLE COLLECTION AND MEASUREMENT OF WEIGHT**

Weight maintenance was used as an index of volume stability to evaluate the graft take previously. Ninety days after implantation, all rats were killed in an ether jar, and the fat grafts were harvested under an operating microscope by a surgeon blinded to the treatment groups. Harvested fat grafts were washed, blotted dry, and weighed (Figure 2F). Postgrafting tissue weights were divided by preimplantation tissue weights, and the percentages of graft survival rates were obtained.
HISTOLOGIC EVALUATION

Harvested grafts were fixed in formalin, 10%, embedded in paraffin, sectioned, and stained with hematoxylin-eosin. Multiple fields of each section were viewed to evaluate the histologic characteristics of adipose tissue. The percentage rate of lipid-extracted area inside the adipocyte membrane compared with the total microscope viewing area was calculated. This ratio was defined as the relative adipocyte index.

Microvascular density was determined in hematoxylin-eosin–stained samples. A pathologist, blinded to groups, counted microvascular structures in 12 microscope areas (at original magnification ×20).

STATISTICAL ANALYSIS

All data were analyzed using SPSS statistical software for Windows, version 11.0. Weight maintenance, fibrotic area ratio, and
relative adipocyte index differences among the groups were tested with 1-way analysis of variance (ANOVA) using post hoc test of least significant difference. Comparisons of 2 groups were tested with t test ($P < .01$ for nonparametric tests and $P < .05$ for parametric tests). Data from a blood vessel count were analyzed using Group differences were tested with 1-way ANOVA. Double group comparisons were made with Tukey test.

RESULTS

All animals tolerated the operations well. There was no evidence of any infection, hematoma, or seroma. Weight differences, before and after the procedure, were not statistically significant ($P > .05$). Explanted grafts were easily dissectible. In group 3, grafts were firmer than in other groups. Other groups were softer to the touch.

WEIGHT MAINTENANCE

Dry weights of the sample grafts were measured. The weight averages of groups 1 and 2 (treatment), and group 3 (an empty microsphere added to the graft) were significantly higher than that of the control group ($P < .05$). The means (SDs) in increasing order were as follows: control group, 44.0% (2.2%); group 3, 107.0% (26.0%); group 2, 141.6% (12.8%); and group 1, 125.3% (9.9%). There was no difference detected in between groups 1 and 2 ($P = .34$) (Figure 4).

HISTOLOGIC AND HISTOMORPHOMETRIC CHARACTERISTICS

Histologic evaluations revealed significant differences between the groups. The fibrotic area ratio was defined as the fibrotic area compared with 1 microscope area ($P < .05$). Group 3 had a higher fibrotic area ratio than the control group ($P < .05$) (Figure 5).

The mean (SD) relative adipocyte indices were 80.6% (4.0%) in the control group; 94.5% (1.5%) in group 1; 88.6% (2.2%) in group 2; and 61.3% (5.7%) in group 3. Groups 1 and 2 showed a significant difference compared with the other 2 groups ($P < .05$) (Figure 6). The mean (SD) vessel counts per microscope area were 24.42 (7.74) in group 1, 18.86 (4.99) in group 2, 7.92 (3.47) in group 3, and 6.59 (2.95) in the control group. Statistical evaluations revealed that group 1 had a significantly higher density than all other groups ($P < .001$) (Figure 7). Group 2 had a significantly higher density than group 3 and the control group ($P < .001$). Group 3 and the control group showed no difference ($P > .05$).

Vascular endothelial growth factor is a glycoprotein that induces proliferation, migration, and differentiation of endothelial cells. VEGF-A, with 165 amino acids, is the most prominent form and widely used. This isoform binds to the proteoglicans and heparin in the extracellular matrix medium providing the longest half-life. Nishimura et al showed that VEGF levels significantly rise at about the seventh day after grafting. Grafts are nourished via diffusion for the first 4 days. Fat necrosis occurs until revascularization is accomplished. However, apoptosis continues. After the fourth day after grafting, progressive microvascular invasion starts. Aiko et al, Hutley et al, and Fukumura et al suggested that endothelial cells have promotive effects on preadipocytes in a paracrine manner via either VEGF or some other soluble factors. Elcin et al used VEGF in calcium alginate microspheres and showed that vascular density increased in the rat dorsum. We preferred to use VEGF in an alginate microspheres model because alginate is inexpensive, easy to prepare, and, most important, was previously shown to increase the subcutaneous vascularity of the rat dorsum.

The rat dorsal area was used for the recipient bed model. This model has several advantages. The subpapnicular area in rats is a relatively avascular plane, which...
eases the operative process. Also, this plane does not contain inherent fat tissue. Thus, the only fat you measure is the fat you engraft. Measurement of the fat weight is safe and reliable. Mouse models, with their similar anatomy, could also be used. However, the inguinal fat pads of mice are not large enough for manipulation.

Because low vascularity of the recipient bed seems to be an important factor for fat graft necrosis, placing the graft in a well-vascularized bed is a logical strategy to increase graft take. Some authors suggest that muscle tissue is the best recipient site for fat grafts because it has a high vascular supply. Baran et al studied fat grafts in an animal model in which a silicone sheet was placed in the recipient bed 3 weeks prior to engraftment, reminding us of the “delay phenomenon.” They showed that lobular fat grafted in a silicone sheet capsule had satisfactory results.

Figure 5. Photomicrographs of fat grafts from each group of rats (hematoxylin-eosin, original magnification ×20). Group 1, preconditioning, in which vascular endothelial growth factor (VEGF) was applied prior to the fat grafting; group 2, fat grafting and VEGF; group 3, grafting and empty microsphere; and control group. A and B, Group 1. Note increased adipocyte viability and vascular structures. C and D, Group 2. Note increased adipocyte viability. E, Group 3. Note dense fibrosis and mononuclear cell infiltration. F, Control group. Note less fibrosis than in group 3, increased fat necrosis, and mononuclear cell infiltration.
even 1 year after surgery. Less necrosis and calcification and fewer cysts were also seen when the fat graft was placed in muscle. Our results, indicating that preconditioning the recipient bed with VEGF has better survival of the fat graft, agree with those in the current literature.

In some other studies the fat graft was manipulated. Yuksel et al\(^\text{20}\) used insulin, insulinlike growth factor-1 (IGF-1), and basic fibroblast growth factor in a poly (lactic-co-glycolic-acid)–polyethylene glycol (PLGA/PEG) microsphere delivery system. They reported successful results consistent with ours.\(^\text{22}\)

Microvessel density of the samples revealed a significant difference between groups 1 and 2 and other groups. Yi et al\(^\text{12}\) transected VEGF gene with an adenovirus vector and transplanted fat grafts to nude mice to evaluate the effects of VEGF on fat grafts. They showed that the VEGF-transfected group had higher microvessel density. Our results are consistent with those of Yi et al.\(^\text{12}\)

Weight maintenance differences between the groups suggest that VEGF may increase weight of the graft by the 90th day after grafting. However, the increased weight of group 3 (in which an empty microsphere was added to grafting) is surprising. We speculate that this may be due to increased fibrosis in group 3 as revealed by histologic examination. Another possibility is that alginate oligosaccharides may increase the weight on its own. This postulation is based on the observation by Kawada et al.,\(^\text{23}\) who showed that alginate oligosaccharides increased the mitogenic and migratory effects mediated by VEGF.

Group 3 had more fibrotic area than other groups. This finding may elucidate the percentage of the graft weight increase in group 3. Fat necrosis may also contribute to fibrosis.

One shortcoming of this study is the small number of animals in the sample. Local regulations for animal welfare recommend the least number of animals for any experimental setting. However, this limits the sample. There is 1 more issue that needs to be clarified before VEGF can be used clinically in human subjects. That is oncological safety. Antiangiogenic treatment is one of the treatment aims of the new era.\(^\text{24}\)

These results suggest that sustained supplementation of VEGF in the early stages of grafting would increase the viability of the adipocytes. Moreover, preconditioning of the recipient bed with VEGF containing alginate microspheres may increase the viability of the fat grafts. Alginate carrier may be a promising agent for microvascular manipulation.

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