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original article

Contrast-enhanced ultrasonography evaluation after autologous fat grafting in scar revision

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SUMMARY: **Contrast-enhanced ultrasonography evaluation after autologous fat grafting in scar revision.**

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Aim. *Fat transfer is commonly used to fill loss of volume in depressed scars caused by trauma, deep burns or surgery. The aim of the study is to investigate the degree of fat graft take through evaluation of the microcirculation of grafted autologous adipose tissue using contrast-enhanced ultrasonography.*

Patients and method. *From 2010 to 2012 at the Department of Plastic and Reconstructive Surgery of the Traumatological Center in Turin, a study population was selected from patients with surgical indications for autologous fat transfer for scar correction. For each surgical procedure patients underwent a clinical and sonographic evaluation before and after intervention (at 1 month and 3 months).*

Results. *Out of a total of 28 interventions, 24 showed a good result; defined as improvement of the scar, and confirmed by the presence of vascularization in the transplanted tissue. In 4 cases, there was a lack of blood supply at the first evaluation but an initial good clinical scar correction. The absence of blood vessels was confirmed at 3 months accompanied by complete resorption of the transferred fat with a failure of good clinical outcome.*

Conclusion. *Contrast-enhanced ultrasonography was able to evaluate the microvasculature of adipose tissue after fat transfer. Due to this characteristic, it allows to monitor and predict the take of adipose tissue and provide realistic and early information on the clinical outcome of fat transfer.*

KEY WORDS: Autologous fat grafting - Contrast-enhanced ultrasonography - Scar - Trauma - Surgery.

Introduction

Autologous fat transfer has recently been used to fill defects of soft tissues caused by trauma, deep burns or surgery. Adipose tissue has been shown to be an accessible source rich in mesenchymal stem cells (MSCs). These present an innovative therapeutic opportunity in many clinical fields including plastic and reconstructive surgery, especially for their ability to enhance mechanisms of repair in many tissues (1). MSCs are particularly interesting because they have the ability to self-renew and differentiate into many different cell types when cultured, particularly into cells of mesodermal origin, such as fibroblasts, osteoblasts, chondrocytes and adipocytes (2).

On this basis, fat grafts should be considered as ideal fillers, providing autologous and completely biocompatible tissue. Fat is available in sufficient quantities in the majority of patients, even very thin individuals, with low associated donor site morbidities. It integrates naturally in the recipient site, can be removed if necessary and is potentially permanent (3). The main limitation is an unpredictable graft resorption, which ranges from 30% to 80%, probably as a result of ischemia and lack of angiogenesis (4). Therefore multiple grafting procedures are often necessary to achieve reasonable results (5, 6).

Loss of transplanted fat tissue probably results from lack of adequate vascularization. This hypothesis is supported by studies demonstrating that larger and thicker fat grafts undergo relatively more resorption (7, 8), as considerable numbers of mature adipocytes undergo apoptosis within the first few days after transplantation due to their low tolerance for ischemia (9). For these reasons, success of autologous fat grafting is highly dependent

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on the vascularity of the recipient site. Moreover, high rates of resorption have also been attributed to traumatic handling of the graft during harvesting and transplantation; therefore, caution during fat harvesting and injection of small amounts of adipose tissue in multiple planes are important factors responsible for graft take (6, 10-12). The role of anesthetics should be considered, since the fat graft is exposed to local anesthetics at both the donor and recipient sites. Some clinical studies have shown that lidocaine potently inhibits glucose transport and lipolysis in adipocytes, as well as their growth in culture. The use of local anesthetics may halt their metabolism and growth (13-15).

In fact, there is no clarity on the percentage of fat cells surviving after harvesting; moreover, follow-up usually is subjectively based on clinical pictures.

A few studies tried to measure results of adipose transplantation using radiological investigations in terms of volume and morphology; however, the pathophysiology of graft take remains quite unknown.

The purpose of this paper is to investigate the degree of graft survival/ resorption through evaluation of the microcirculation of grafted autologous adipose tissue using contrast-enhanced ultrasonography (CEUS). The goal of this study is to evaluate the effectiveness of autologous fat graft take using this radiologic modality.

Patients and methods

Study design

From November 2009 to March 2013, a prospective non-controlled non-randomized study was set at the Department of Plastic and Reconstructive Surgery of the Traumatological Center in Turin. The inclusion criteria outlined in Table 1 were used to select a study population from patients with surgical indications for autologous fat transfer for scar correction.

For each surgical procedure, patients underwent a clinical evaluation and photographic documentation before and after operation (at 1 and 3 months postop).

Table 1 - CRITERIA FOR PATIENT SELECTION IN FAT TRANSFER PROCEDURES.

INCLUSION CRITERIA	EXCLUSION CRITERIA
Age > 18 years	Age < 18 years
Presence of scar depression	Aesthetic purposes
Presence of scar adherence to deep layers with	Cardiovascular disease
Scar deformity	Diabetes mellitus
Burn or traumatic or surgical scar	BMI < 20

Scar areas to be treated were studied by CEUS at the Radiology Unit of Traumatological Center at baseline, and at 1 month and 3 months after surgery.

The study followed the ethical rules for human experimentation stated in the 1975 Declaration of Helsinki. Approval by our institutional Ethical Committee was obtained.

Surgical technique

Informed consent for surgery was obtained from all patients. Surgery was performed under either general anesthesia or local anesthesia with sedation. A short-term antibiotic prophylaxis was given with 1g of amoxicillin/clavulanic acid or 2g of ceftriaxone.

The donor site location was chosen on the basis of fat availability. The abdomen, lateral hips and medial regions of the knees were mostly selected.

Procedures were performed by three different operators using a standardized surgical technique in order to minimize bias.

Without tumescent infiltration, fat was harvested in a "dry" way. A Coleman cannula was connected with a Luer Lock to a 10 ml syringe under negative suction. The fat obtained was centrifuged at 3000 RPM for 3 minutes, then separated by oil and blood residues and the injectable lipoaspirate was collected into 5 ml Luer Lock syringes ready to be injected. Fat was injected using Coleman cannulae through 2 mm cutaneous incisions. Choice of cannula [straight or curved, blunt or cutting (sharp dovetail)] was made depending on the amount and shape of filling desired and the degree of scar adherence to deep layers. The injection technique was done according to Coleman's experience: transfer of small amounts of fat in multiple steps and layers (6).

Ultrasonography

All patients underwent ultrasound examination at baseline (B-mode) with color module Power-Doppler (PD) and during intravenous administration of a second generation ultrasound contrast agent. All patients have given their informed consent for the invasive procedure. In case of suspected allergy, the patient received prophylactic anti-allergic therapy. All radiologic examinations were performed by the same operator.

Examinations were performed with ultrasound MyLab 70 and MyLab Twice (Esaote®, Genoa, Italy) equipped with high-frequency linear probe (7.7-12 MHz) and contrast-specific software CnTI with a low mechanical index.

The ultrasound contrast agent used (SonoVue®, Bracco, Italy) consists of sulphur hexafluoride microbubbles, with a diameter of 3-5 micrometers (smaller than a red blood cell), and stabilized by an outer elastic shell. Due to their small size, once these microbubbles are introduced into the blood stream, they remain intravascular and do not spread to extravascular spaces. Therefore, unlike other contrast agents used in computed tomography or magnetic resonance, the microbubbles assume the characteristics of a blood-pool contrast agent. Furthermore, SonoVue® is able to cross the alveolar membrane and is predominantly eliminated through respiration in 15-20 minutes. Ultrasound contrast agent is very easy to handle, has a low incidence of allergic reactions, and can be used in patients with kidney or liver disease (16).

Inside an ultrasound field, microbubbles undergo rhythmic phenomena of compression and expansion and they send out a harmonic response different from the one sent out by surrounding tissues. Using special software with low mechanical index (therefore unable to destroy microbubbles), it is possible to view the passage of contrast real-time within vessels of small dimensions. A signal sent out from microbubbles corresponds to the presence of vascularization.

The operator measured the thickness of the subcutaneous tissue in the treated area during the baseline examination with ultrasound B-mode. In order to have a reproducible measurement in subsequent examinations, the operator looked for precise anatomical landmarks or, in case of large areas, marked the point with a dermographic pencil, saving a digital image (Figure 1). Then the operator proceeded to study vascularization with Power Doppler despite its limitations to sample flows at low speeds. During the administration of ultrasound contrast agent it was possible to show the presence of vascularization by recording short videos (less than 40 sec). A normally perfused subcutaneous tissue shows small vessels with bush-like pattern and a uniform but non-dense distribution.

Results

Nineteen patients satisfied the criteria described in Table 1 and were enrolled in this study. A total of twenty-height surgical procedures of fat grafting were performed by three different operators. Six patients underwent surgery twice and one patient underwent surgery four times.

Characteristics of the study population are reported in Table 2.

The patient group consisted of 31% males and 69% females aged from 19 to 59 years (mean 40 years). Treated areas were usually pathological scars in complete remission with a loss in volume and presence of adherence to underlying tissues (Figure 2).

The most frequently treated anatomical area was the face (21%), followed by the breast (14%), chest wall (14%) and neck (10%).

There were no acute complications (i.e., infections or hematomas) after fat grafting.

Among 28 interventions tested at 1 month and 3 months with a clinical check-up and sonographic examination, 24 showed a good result consisting of improvement of scar quality together with filling of volumetric defects which was confirmed by the presence of vascularization of the transplanted tissue on examination using CEUS. Fat graft take was confirmed in the first month check-up as well as the second one at three months (Figure 3).

On the contrary, in 4 cases there was a lack of blood supply at the first evaluation at 1 month postoperatively. Notably, an initial good clinical result was detected in

Fig. 1 - Large scar with important soft tissue gap; in order to have a reproducible measurement in subsequent examinations the operator looked for precise anatomical landmarks and signed the point with a dermographic pencil.

TABLE 2 - CHARACTERISTICS OF POPULATION STUDY.

Case	Patient	Age	Sex	Site	Operator	CEUS-0	CEUS-1m	CEUS-3m
1	P M	41	F	Chest	C	+	+	+
2	M G	51	F	Face	A	+	+	+
3	M G	51	F	Face	A	+	+	+
4	P M	42	F	Chest	C	+	+	+
5	R T	42	F	Breast	B	+	-	-
6	R T	42	F	Breast	B	+	+	+
7	S M R	53	F	Foot	C	+	+	+
8	T F	31	F	Forearm	C	+	+	+
9	M S	29	F	Face	A	+	+	+
10	M S	30	F	Face	A	+	+	+
11	M A	19	M	Leg	C	+	+	+
12	G D	41	F	Foot	C	+	+	+
13	R E	41	F	Ankle	C	+	-	-
14	V E	50	F	Thigh	B	+	+	+
15	V D	19	M	Chest	A	+	+	+
16	V D	20	M	Chest	A	+	+	+
17	B M	30	M	Leg	B	+	-	-
18	C S	19	M	Hand	C	+	+	+
19	A D	42	F	Breast	C	+	+	+
20	A D	42	F	Breast	C	+	-	-
21	N M R	43	F	Buttock	C	+	+	+
22	U L	30	M	Thigh	C	+	+	+
23	M L	59	F	Neck	A	+	+	+
24	N M	50	M	Hand	A	+	+	+
25	N M	50	M	Hand	A	+	+	+
26	M G	52	F	Face	A	+	+	+
27	F L	49	F	Neck	C	+	+	+
28	M G	53	F	Face	A	+	+	+

CEUS: contrast-enhanced ultrasonography; A: first operator; B: second operator; C: third operator.

these patients. However, at the three-month check-up, failure in clinical scar improvement was observed, accompanied by complete resorption of injected fat and confirmed by the absence of detectable vessels by sonographic examination (Figure 4).

Discussion

Fat transfer offers a natural alternative to alloplastic implants for corrections of localized areas of volume loss. However, despite the fact that fat resorption is a well-documented phenomenon, our ability to evaluate it objectively remains limited (17).

The remodeling and regeneration of newly grafted adipocytes is a dynamic process determined by the presence of a nearby nutritional host source and fine regulated by mesenchymal stem cells (18).

Eto et al. describes three zones of healing among the newly grafted fat located in a host bed: the necrotic, the regenerating and the surviving zones, respectively positioned centrally to peripherally in the grafted tissue. While all cells die within the necrotic zone, stem cells survive within the regenerating zone and function to replace dead adipocytes with new cells. Both adipose-derived stromal stem cells and adipocytes survive within the peripheral surviving zone. Thus, it is not just the initial volume of fat transferred that determines the final volume, but also the complex interactions between multiple cell types, including adipose-derived stromal stem cells, viable

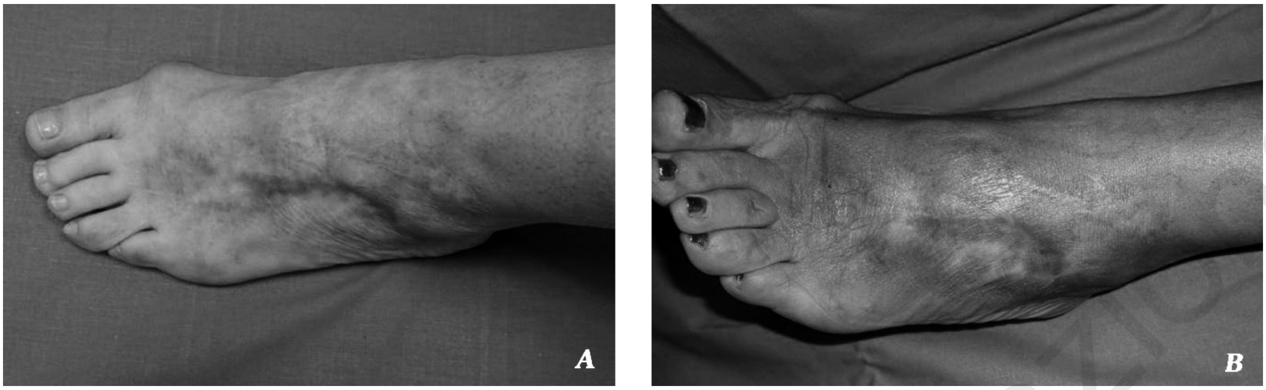

Fig. 2 - Successful case of autologous fat transfer. A) Traumatic scar of the dorsum of the foot with strong adherence to the deep layers and loss of volume. B) Clinical improvement of scar quality, with release and volume restore.

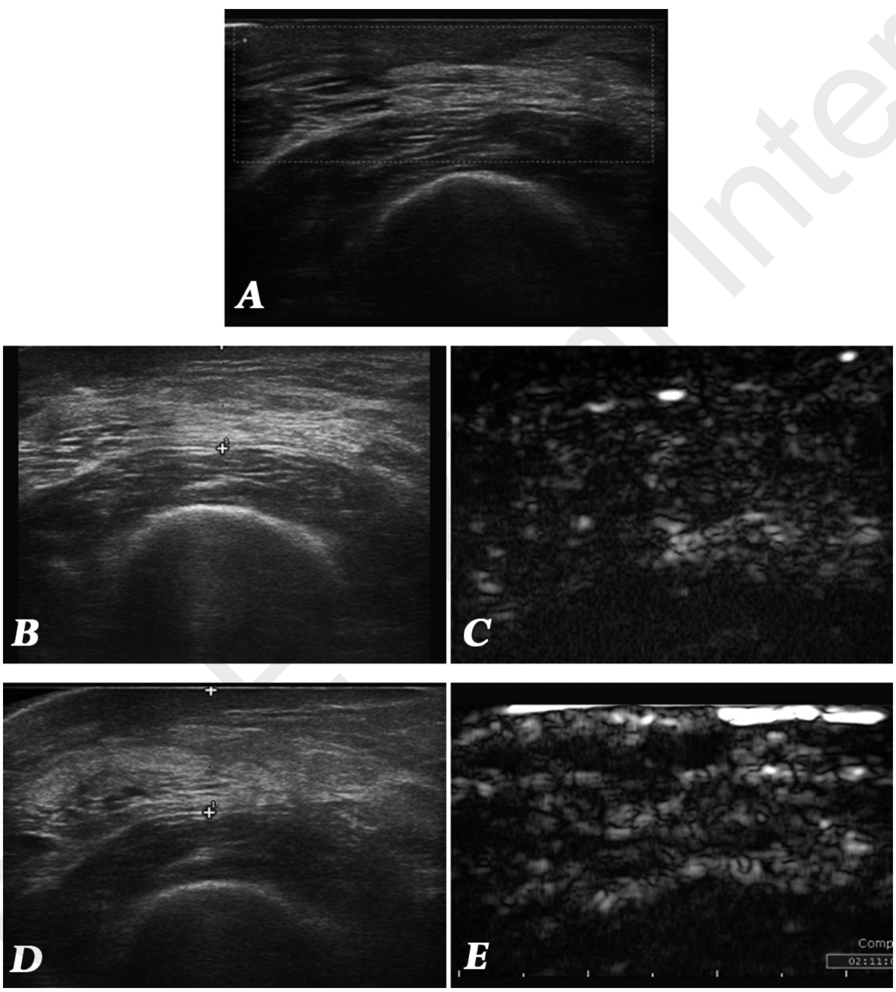

Fig. 3 - Successful case of autologous fat transfer documented by sonography and contrast-enhanced ultrasonography. A) Thickness of subcutaneous tissue pretreatment. B-C) Increase of thickness (B) and homogenous vascularization (C) 1 month post autologous fat transfer. D-E) Fat graft stabilized 3 months post treatment.

adipocytes and necrotic adipocytes that stimulate maintenance of a set volume as determined by the original composite of tissue transferred into the wound bed (19).

The distribution patterns after fat injection remain entirely unknown. Spear and Coleman have reported the use of two-dimensional photography to evaluate the degree of improvement following fat grafting, but this approach has significant limitations (20).

Attempts to integrate more objective measuring tools such as water displacement, thermophilic casting and

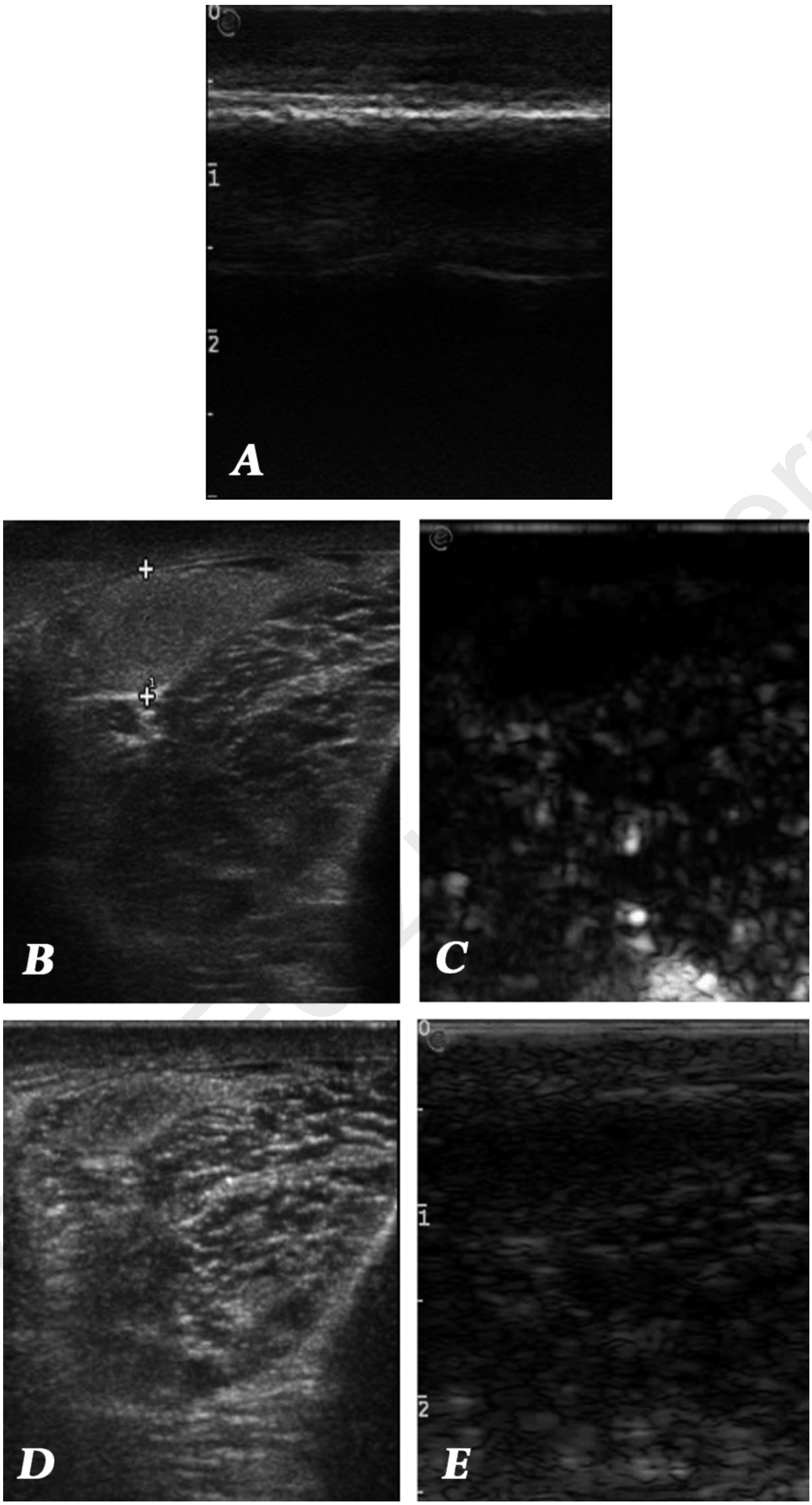

Fig. 4 - Unsuccessful case of autologous fat transfer documented by sonography and contrast-enhanced ultrasonography. A) Thickness of subcutaneous tissue pre-treatment. B-C) Increase of thickness (B) with absence of vascularization after contrast injection (C) 1 month post autologous fat transfer. D-E) Fat graft resorption (D) with complete losing of vascularization (E) 3 months post treatment.

magnetic resonance imaging-constructed three-dimensional models have been limited because of their cumbersome and time-consuming nature (21,22). Therefore, no accurate and reproducible tool exists to quantify the outcome after fat transfer.

The B-mode ultrasound with linear probe from 3 to 12 MHz is a suitable modality for the study of injected fat because it is able to distinguish it very well from the surrounding subcutaneous tissue.

It allows the analysis and monitoring of the fat thickness obtained and is also able to detect any complications such as formation of fatty cysts and eggshell calcifications (23). B-mode ultrasound combined with contrast-enhanced injection can help the clinician to study deep and superficial vascularity.

In our series CEUS was able to demonstrate an absence of vessels within the fat graft in 14% of all surgical operations, with consequent failure in its host integration. These data were strongly correlated with our long term follow-up clinical findings; in fact, in these 4 cases there was no clinical improvement of scar characteristics. Moreover, we noted that CEUS has the ability to predict what the long-term result will be as early as the first month postoperatively while it is difficult to clinically predict graft failure at this time point.

In all our cases, positive and negative, at the third month CEUS has always confirmed what the first evaluation showed: the presence of vascularization stabilizes and steadies the fat graft whereas the absence of angiogenesis causes its progressive reabsorption.

Conclusion

On the basis of our experience, CEUS is a reliable and reproducible method for determination of the microvascular volume of adipose tissue graft take. Tobin (24) initially described this modality in the analysis of abdominal subcutaneous adipose tissue, which possesses the ability to recruit capillaries in postprandial period. Vascular volume in the studied tissue is expressed as an increase in signal intensity from baseline to the first plateau phase. In conclusion, these vascular signal recordings can objectively quantify autologous fat graft take. In the post-operative follow-up, monitoring the vascularity of transplanted adipose tissue can be an index of a graft survival, thereby predicting the outcome. Therefore CEUS can provide information on graft take in the early postoperative period that is not otherwise clinically detectable, thus helping plastic surgeons to manage failure in fat graft take early on.

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