Management of Vocal Fold Scars by Concurrent Nanofat and Microfat Grafting

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Abstract: Vocal fold scarring is the cause of severe dysphonia and represents a therapeutic challenge; dysphagia can also be present in case of soft tissue defect due to previous oncological surgery. The ideal surgical solution should concurrently provide vocal fold augmentation and re-establishment of tissue elasticity. Nanofat technique has given so far promising results in remodeling skin scars and improving tissue pliability. The present paper describes for the first time the use of nanofat injected into the vocal fold cover for pliability restoration, combined with traditional microfat for vocal fold augmentation. Seven patients (aged 23–77 years) affected by severe dysphonia, related to extensive vocal fold scarring (3 of them were also affected by dysphagia for liquid consistencies), underwent a single procedure of concurrent microfat and nanofat vocal fold injection under direct microlaryngoscopy in general anesthesia. Results were evaluated by objective outcome measures and auto evaluation performed by questionnaires concerning the phonatory and swallowing efficiency. The voice quality and the perceived swallowing capability of all patients improved after surgery and are stable at follow-up (4–8 months). The reported preliminary data show that nanofat, due to its regenerative potential related to adipose-derived stem cells and growth factors, can be a promising adjunct to traditional fat augmentation to improve elasticity of the delicate multilayered structure of the vocal fold and to enhance its vibratory capabilities. Further experience on a wider number of patients and long-term follow-up are necessary to confirm the validity of this technique.

Key Words: Dysphonia, fat grafting, glottic incompetence, nanofat, tissue engineering, vocal fold scarring

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Dysphonia due to vocal fold scarring is a challenging condition for the otolaryngologist; injection laryngoplasty[1] is the most common therapeutic solution but results are still controversial.

The vocal folds are the source of voice sound, but they do also play a prominent role as a sphincter in protecting the lower airway from the entrance of food and regulate the inspiratory and expiratory airflow. The efficiency of their closure is crucial for voice production and for the sphincteric function; the defective closure is termed *glottic incompetence* or *glottic insufficiency*. The etiology can be due to a congenital or acquired soft tissue defect or to vocal fold paralysis. Glottic incompetence is the cause of a breathy voice and of vocal fatigue; often the patient experiences also a swallowing impairment, especially for liquid consistencies, due to defective protection of the airway, and also shortness of breath during speech.

Voice rehabilitation is helpful in treating the minor cases of glottic insufficiency but in more severe cases surgical treatment can re-establish glottic competence by medializing and augmenting the volume of the affected vocal fold. Several implants and biomaterials have been proposed to obtain vocal fold augmentation.[1] They can be injected also on an outpatient basis, under local anesthesia;[2] however, local complications can occur, including foreign body reactions, extrusion, and reduced tissue elasticity, with possible permanent worsening of dysphonia.[3] The autologous tissues utilized are mainly fascia[4] and fat.[5–9] The use of fat was introduced since the 1990s,[10,11] but, due to resorption tendency,[12] fat was not widely used for permanent vocal fold augmentation, and only a few authors have reported consistent long-term outcomes with fat.[9,13]

Recently, a novel way to process the lipoaspirate has been proposed and named *nanofat*[14] in contraposition to the term of *microfat* commonly used to define the fat microparcels obtained by liposuction with a cannula of small diameter.

It is known that the lipoaspirate contains fat cells, adipose-derived stem cells (ADSCs), and growth factors.[15,16] It has been claimed that the scar-remodeling effect of fat grafts may be related to ADSCs.[17] The nanofat is obtained emulsifying the lipoaspirate and its use has been proposed for skin rejuvenation,[14] but also to improve results in the treatment of scars,[14,18] as it can be injected into the dermis by thin needles. It has been demonstrated that ADSCs and growth factors are still present in nanofat and they are supposed to favor the improvement of tissue elasticity.[14,18]

Treating vocal fold scarring represents a challenging goal in the field of laryngology. Vocal fold pliability is a critical condition for an efficient voice production. The vocal fold has a complex multilayered structure, and integrity of the lamina propria is crucial to allow fluctuation of the superficial layer as a mucosal wave on the deeper layer. If a scar impairs this fluctuation, the voice will be perceptually altered, and the patient will perceive a high degree of fatigue and respiratory effort to sustain the voice sound. Freeing of the mucosa technique[19] has been proposed to improve this condition but unfortunately scarring adhesions tend to recur: Replacing the superficial gliding tissue of the vocal fold has been addressed by several clinical and experimental studies,[20] often without achieving a significant improvement.

Based on the favorable results reported so far by using nanofat for the treatment of skin scars and for superficial face wrinkles,[14,18] we utilized the technique for the management of vocal fold stiffness due to scarring. The aim of the present paper is to describe the preliminary results achieved in treating vocal fold scarring by means of the nanofat technique combined with microfat injection.

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PATIENTS AND METHODS

Patients

A total of 7 patients, aged 23 to 77 years (mean age 51), were included in the present study (see Table 1). All patients were affected by severe dysphonia related to scarring of the vocal folds.

The soft tissue defects of the vocal folds were either congenital (sulcus vocalis; 2 cases) or acquired, due to sequelae of excision of vocal fold malignancies (3: 2 sequelae of endoscopic cordectomy, 1 sequela of open partial horizontal laryngectomy), or benign lesions (2). In the 5 patients with acquired lesions, the time elapsed from the primary surgery ranged from 1 to 6 years (mean: 2.6 years). All patients had undergone voice therapy prior to surgery and surgical treatment was proposed because results were unsatisfactory.

Voice rest was prescribed for the 24 to 48 hours following surgery. Patients were discharged 24 hours after the operation.

Patients were assessed 7 days and 1 month after surgery, then every 3 months. Follow-up is 4 to 10 months.

Laryngeal Assessment and Voice Evaluation

A multidimensional assessment was utilized to ascertain the preoperative condition and to evaluate voice outcomes. Patients were assessed preoperatively, 1 week, 1 month after surgery, and then every 3 months, by the following means:

1. Videolaryngostroboscopy with a flexible fiberscope or a 70° rigid fiberoptic endoscope allowed us to ascertain the site and severity of the glottic gap, to visualize vocal folds vibration abnormalities, to plan the injection sites, and to objectively assess posttreatment changes
2. Perceptual voice evaluation, by means of the GRBAS scale[21] which subjectively scores the grade of dysphonia (G), roughness (R), breathiness (B), asthenicity (A), and strain (S). The voice samples were computer-recorded while the subject produced a sustained /a/, repeated single words and sentences, and during conversation. The recordings were subsequently evaluated by 3 experienced independent listeners (2 speech therapists and 1 phoniatrician) and scored from 0 (= normal) to 3 (= severe disturbance), decimals were also computed. The G, R, and B scores were computed for outcome evaluation.
3. Maximum phonation time measurements, obtained during the production of a sustained vowel /a/ at a comfortable pitch and loudness; the best of 3 consecutive trials was considered. This is considered the simplest way to obtain an indirect measure of glottic closure. It is an aerodynamic parameter which is also influenced by the patient's vital capacity.
4. The Voice Handicap Index questionnaire (VHI),[22] administered for the self-assessment of perceived voice-related disability. Voice Handicap Index questionnaire is a 30-item test with 3 subscales that measure the functional, physical, and emotional aspects of the handicap caused by voice impairment. The subscale scores range from 0 to 40, and the total score ranges from 0 to 120; a higher score indicates a greater degree of disability. A score below 18 is considered to be within normal limits.
5. The EAT 10 questionnaire[23] used in the 3 patients with sequelae of oncological surgery to assess the swallowing impairment due to glottic incompetence.

Fat Harvesting and Processing

The procedures were performed with the patient under general anesthesia, which is necessary to allow access to the vocal folds by direct rigid microlaryngoscopy. Fat was harvested from the abdomen. After infiltration with 10 cc of lidocaine and epinephrine 1:100,000, harvesting was performed by means of a 10 cc Luer-lock syringe connected to a 2 or 3 mm cannula. To provide negative pressure the plunger of the syringe was retracted and maintained in that position by means of a towel clamp. The lipoaspirate was processed according to Coleman.[24] After separation from the blood and oily components, half of the fatty layer was transferred to a 3.0 mL syringe using a Luer-lock to Luer-lock adapter, while the other half was emulsified by shifting it between two 10-cc syringes connected to each other by a Luer Lock 3-way stop cock connector, according to Tonnard et al.[14] After 30 passages the fat was more fluid as adypocites were broken during the procedure. No filtration was performed.

Injection Procedure

The patients' vocal folds were exposed by direct microlaryngoscopy and palpated to better identify scarred areas. Fat parcels were placed using a lipoinjection handle (Medicon Instrumente, Tuttlingen, Germany) with a 21-gauge, 22-cm-long bayonet needle. First, we injected the refined microfat parcels deeply in the vocal fold avoiding bolus injection and trying to maximize the contact of the grafts with host tissue; therefore, injection was multilayered and at multiple entry points. Afterward the nanofat emulsion was injected in the most superficial layer of the vocal fold in the scarred tissue with the aim of regaining pliability and elasticity and to restore the vibratory capability. Butterfly needles (23 and/or 25 gauge) connected to a 3 cc luer lock syringe were used for nanofat injection. The needle's wings were cut to allow easy manipulation of the needle itself by means of an alligator forceps designed for microphonosurgery, the needle was bent as needed to facilitate the injection in the scarred tissue.

TABLE 1. Demographics of the Seven Treated Patients

Cases	Gender	Age (Yrs)	Etiology	FU mo	G Pre	G Post	R Pre	R Post	B Pre	B Post	VHI Pre	VHI Post	MPT (s) Pre	MPT (s) Post	EAT 10	EAT 10
1.	M	54	Oncol	10	3	1.8	3	1.8	3	1.8	49	15	6	12	17	2
2	M	57	Oncol	8	3	1	2	0.8	3	1	91	24	2	4	31	7
3	F	46	Benign	8	2	1.5	2	1.5	2	1	45	36	6	9	—	—
4	M	23	Congenital	6	2.4	1.8	2	1.5	2.3	1	51	31	8	12	—	—
5	M	46	Congenital	6	2.5	1.4	1.2	0.8	2	1.2	56	19	8	15	—	—
6	F	55	Benign	5	2	1.2	0	0	2	1.5	48	44	6	7	—	—
7	M	77	Oncol	4	3	1.4	0	0	3	1.4	55	36	4	7	1	0

Pre- and postoperative values of the considered variables are reported for each patient.

B, voice breathiness; Benign, previous surgery for benign lesion; Congenital, congenital scarring; EAT 10, EAT 10 questionnaire; FU, follow-up; G, grade of dysphonia; MPT, maximal phonation time; Oncol, previous oncological surgery; R, voice roughness; VHI, voice handicap index.

It is difficult to quantify the amount of grafted fat exactly, because some of it oozes out while removing the needle, but a total of 0.5 to 2 cc of microfat and nanofat was injected in the scarred vocal fold.

Statistical Analysis

Data were collected by chart reviews of prospectically recruited patients. The Wilcoxon matched-pairs signed rank test was used to compare the data gathered before and after surgery. P values less than 0.05 were considered to be significant.

RESULTS

The postoperative course was uneventful. None of our patients experienced postoperative obstructive symptoms. No signs of infection at injection sites were found.

Videolaryngoscopy demonstrated an improvement in glottic closure in all 7 cases.

Voice quality improved after surgery in all patients, as shown by the considered variables (see Table 1). The perceptual evaluation demonstrated a significant improvement of the global grade of dysphonia and of voice breathiness (B) ($P = 0.01$), roughness was also improved but not significantly. Maximum phonation time was significantly longer at the postoperative follow-up, proving that a more efficient vocal folds closure was achieved during phonation ($P = 0.01$). Voice Handicap Index scores demonstrated a significant reduction of the perceived handicap related to the voice disorder ($P = 0.015$). The EAT 10 questionnaire was administered to the 3 patients affected by dysphagia for liquid consistencies, after surgery there was a dramatic reduction of the perceived swallowing impairment (no statistical analysis was performed due to the small number of patients).

The voice results stabilized within 3 months postoperatively and remained stable afterward. All patients reported a reduction of effort during voice production and an improvement in their social lives.

Three case studies are presented in Figures 1 to 3.

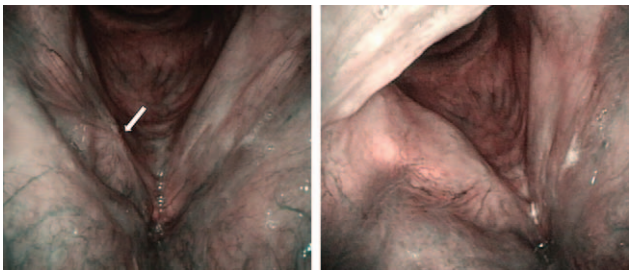

FIGURE 1. Left: extensive scarring (arrow) of the right vocal fold, sequela of oncological surgery (cordectomy for squamous cell carcinoma). Right: 10 months after combined microfat and nanofat injection the surface of the vocal fold is smoother and the whole vocal fold is augmented.

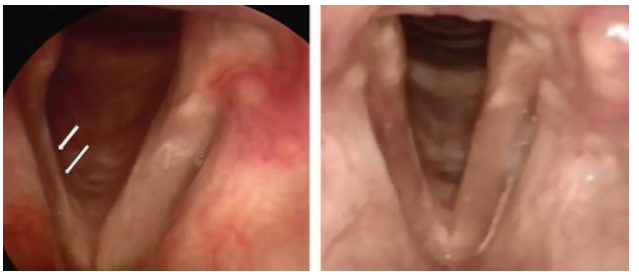

FIGURE 2. Left: the whole left vocal fold (indicated by 2 arrows) is affected by a retracting scar, following cordectomy performed 1 year earlier for squamous cell carcinoma. Right: 4 months after microfat and nanofat injection the contour of the vocal fold is improved and the whole vocal fold is augmented. The patient has a less breathy voice and his swallowing impairment subsided.

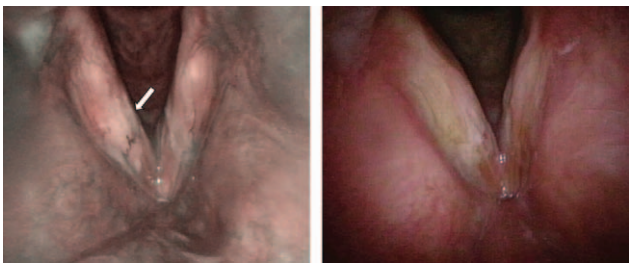

FIGURE 3. Left: a scarred area (congenital defect) causing rigidity of the right vocal fold is indicated by the arrow. Right: result achieved 5 months after surgery, the whole vocal fold is augmented and the retracted cicatricial area has an improved contour.

DISCUSSION AND CONCLUSIONS

The preliminary results of this study support the hypothesis that nanofat injection may have a regenerative role in treating vocal fold scarred tissue. Our previous findings in patients undergoing structural fat grafting of the vocal folds proved the long-term efficacy of the procedure in improving voice quality,[9] Nevertheless, patients affected by soft tissue defects had less favorable results if compared with those undergoing the procedure for vocal fold paralysis. This is an expected finding as in paralyses cases the microstructure of the vocal fold is preserved, and dysphonia is due to the movement impairment. The present study aimed to verify if results can be enhanced utilizing in the same procedure microfat grafting in multiple tunnels[9] to achieve augmentation and nanofat for the treatment of superficial scarring.

Several techniques[1,2] have been proposed so far to medialize the vocal fold affected by scar but most of them just provide augmentation without addressing the main problem, being absence of gliding tissue in the superficial layer of the vocal fold, namely the lamina propria. A few experimental studies were designed for the regeneration of the lamina propria by means of stem cells.[25–28] Purified and cultured stem cells are a very promising tool for achieving tissue regeneration; nevertheless, the cost of the procedure is currently very high, therefore a systematic use in clinical practice of cultured ADSCs seems to be unfeasible, also considering that patients with severe vocal fold scarring might be candidate to more than 1 procedure. A further problem related to the use of laboratory cultured stem cells is the necessity to perform the procedure in 2 steps, as fat harvesting needs to be performed several days or weeks prior to the injection considering the laboratory processing time. This implies higher costs and is unpractical for patients coming from far away. It should also be considered that a much higher quantity of fat is needed to obtain a significant amount of ADSCs. This could be a problem in skinny patients.

Previous laboratory studies[14] demonstrated that nanofat emulsion is rich in ADSCs and growth factors. A technique allowing injecting the scar with an autologous and easily obtainable fluid, with high regenerative potential, is highly desirable as potential advantages are evident. We utilized unfiltered nanofat as described by Jan et al.[29]

The current study has some limitations; first, we did not characterize the constituents of the unfiltered nanofat being used; then, only a small number of patients were treated. A further limitation is the combined concurrent grafting of both microfat

and nanofat; it could be argued that the improvement obtained in our cases might be due to the augmentation obtained by microfat placement. Nevertheless, in the treated patients the fluidity of the nanofat emulsion allowed us to undermine the superficial stiff layer of the pathological vocal folds by thin needles. The re-establishment of more efficient vocal fold vibration was possibly favored by the nanofat regenerative properties.

The future perspective of this study is the application of the described technique to a wider number of patients and a long-term evaluation of the achieved results. Laboratory characterization of the components of the unfiltered nanofat will also be an important goal to better understand its regenerative potential.

In conclusion, tissue engineering techniques are promising tools to re-establish and regenerate the gliding tissue in vocal folds to improve voice quality. The nanofat injection is a simple and straightforward procedure for treating vocal fold scars with a regenerative purpose.

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