Nanofat for Injection Laryngoplasty: A Preliminary Study of a New Substrate☆

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Summary: Objective. "Nanofat" refers to fat further emulsified into 1-μm sizes. It is commonly used in facial plastic surgery. Nanofat increases the release of adipose-derived stem cells. This study tested whether injection laryngoplasty using nanofat resulted in bulking and vibratory effects comparable to using microfat in treating patients with fold atrophy.

Methods. This was a randomized, controlled, single-blind, prospective study of 18 patients with vocal atrophy who underwent transoral lipoinjection using microlaryngoscopy. The control group received bilateral microfat injection. The experimental group randomly received microfat in one vocal fold and nanofat in the contralateral vocal fold. The average volume of fat injected was 0.6 mL on each side. The postsurgery evaluation at 3 months included ratings of stroboscopy and acoustic recordings by blinded expert raters. Vibratory behavior, voice ratings, preVoice Handicap Index-10 (VHI-10) and postVHI-10 score, and Cepstral/Spectral Index of Dysphonia (CSID) were compared.

Results. Significant improvement in the VHI at three-month follow-up was noted in all patients (n = 18 total, 12 experimental, six control). In the subgroup analysis, only the nanofat group significantly improved VHI. Improvements in the CSID were observed in both techniques; however, neither group showed statistical significance. Improved glottic closure was comparable in both groups. Expert raters observed an improvement in the voice quality of nanofat individuals but no change in microfat individuals.

Conclusion. Lipoinjection laryngoplasty with nanofat may be an alternative to microfat in patients with vocal atrophy.

Level of Evidence. II.

Key Words: Nanofat—Microfat—Fat injection laryngoplasty—Augmentation laryngoplasty—Vocal fold atrophy.

INTRODUCTION

Dysphonia is common in elderly individuals, with an estimated prevalence of 47% in a survey involving 117 senior citizens over the age of 65.[1] In a review of patients over the age of 65 presented to a tertiary care center with voice complaints, 24.5% were diagnosed with vocal fold atrophy.[2] Vocal atrophy is an age-related loss of vocal fold muscle mass, resulting in incomplete glottis closure.

Fat injection laryngoplasty fills glottis defects[3] in patients with vocal atrophy or glottic incompetence. The standard fat preparation method uses microfat, which is used in scarring and vocal atrophy rehabilitation.[4] The fat is prepared by cutting it into 1-2-mm sizes for injection using an 18-gauge needle.[5] Although this is effective,[6] technical difficulties may result in variable outcomes. First, there is variability in fat retention, with a loss of up to 70% of grafted fat.[7] Second, injection of the microfat requires large-bore needles. The use of large-bore needles causes tissue trauma and creates large entry holes in the vocal folds. Using large-bore needles may cause fat egress and extrusion during and after the surgery. Fat egress can result in the formation of fat granulomas.

There has been growing interest in using tissue regeneration methods to address vocal fold scarring and atrophy.[8–10] Surgery or injection of stem cells,[11,12] growth factors,[13–15] and extracellular matrices,[16,17] individually or in combination, has shown promise. These regenerative medicine strategies promise to improve vocal function in patients with vocal atrophy.

Nanofat grafting is a different method of fat graft preparation. In this technique, the fat is further reduced to micron size. It has been developed and popularized in facial plastic surgery.[18,19] Although this preparation has a lowered ability for tissue volumization because of a more significant reduction in viable adipocytes, it has shown potential to improve fat graft survival.[20] Additionally, nanofat may enhance fat stem cells compared with microfat. In a study on using nanofat for facial augmentation by Tonnard et al,[18] the nanofat sample was devoid of lipocytes; however, adipose-derived stem cells were still present. Preserving fat-derived stem cells may facilitate tissue regeneration in patients undergoing injection laryngoplasty. The use of nanofat may result in lower variability in absorption than microfat. An additional advantage is that nanofat is delivered through a much smaller cannula.[18] Delivery through a small-gauge needle causes less trauma to the vocal folds.

Despite these theoretical advantages, nanofat is not commonly used for lipoinjection in the larynx.[21]

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This study is a randomized, controlled, single-blinded study of patients with vocal atrophy treated with either microfat or nanofat injection laryngoplasty. We hypothesized that nanofat might result in vocal fold bulking and vibratory effects similar to those of microfat. Short-term (3 months) results are reported in this study. We compared the voice, bulking, and viscoelastic effects of injection laryngoplasty using microfat versus nanofat.

METHODS

Patient selection

This randomized, prospective, single-blind, controlled cohort study was conducted at a single site. The institutional review board at Mount Sinai Hospital approved the study. A total of 18 patients with vocal atrophy were recruited. Recruitment was conducted between August 2021 and July 2023. A senior investigator (PW) identified patients with bilateral vocal atrophy. Treatment options were discussed, including voice therapy, vocal fold injection laryngoplasty, medialization laryngoplasty, or a combination thereof. Those who chose the vocal fold lipoinjections were invited to participate in this study. The patients signed consent forms for randomization purposes.

All patients were required to be over 18 years of age and sign a written consent form for randomization. All patients were required to have been diagnosed with bilateral vocal fold atrophy and have previously undergone voice therapy. Finally, all patients were offered a full range of surgical treatments and decided to pursue vocal fold lipoinjection as the primary and only surgical treatment.

Patients with other vocal fold pathologies, including vocal fold growth, scars, or neurological laryngeal conditions, those medically unfit to undergo elective general anesthesia, and those with prior surgical treatment for vocal fold atrophy were excluded from this study. Vulnerable populations were also excluded.

Patients were randomized into one of the two groups. Randomization was based on the last digit of each patient's social security number. Even numbers were assigned to the treatment arm, and odd numbers were assigned to the control arm. The control group received bilateral microfat injections into each vocal fold. In contrast, the experimental group received a microfat injection into one vocal fold and a nanofat injection into the contralateral vocal fold. Randomization of the side of injection with nanofat was performed to determine the side receiving nanofat using the second-to-last social security number. Equal amounts of fat were injected into each vocal fold in both groups.

Administration of fat

Fat transfer was carried out by microlaryngoscopy under general anesthesia. Intubation was performed using a small endotracheal tube. Suspension laryngoscopy was performed for oral injection laryngoplasty using fat. Microfat injections were administered bilaterally to the control group. Microfat was injected into the vocal folds using a standard 18-gauge needle. The injection site was located lateral to the vocal ligament. In the experimental group, microfat was injected into one vocal fold using the same technique in the control group. An equal volume of nanofat was injected into the contralateral vocal fold using a 25-gauge needle. The injection site for nanofat was also lateral to the vocal ligament. The vocal folds were typically overinjected by 50%. The aim was to place 0.5 cc into each fold. After injection, the laryngoscope was removed, and the patient was then extubated and discharged to home.

Preparation of microfat

A 2-cm periumbilical incision was made to harvest the microfat. The harvested abdominal fat was cut into 1-mm sizes. The 1-mm fat pieces were washed in saline and strained. Methylprednisolone Acetate (Depo-Medrol; 40 mg/cc, one cc) was mixed with fat to stabilize the microfat. The washed fat particles were then placed in an injector gun and injected using an 18-gauge needle.

Preparation of nanofat

Abdominal fat was harvested to obtain nanofat by liposuction using a 3-mm cannula. Tumescent infiltration was performed. A total of 20 mL of fat was harvested by liposuction. The tumescent solution was removed via gravity and decantation. The fat was transferred into a sterile 20-mL syringe and attached to a second 20-mL syringe using a 2.4-mm luer-to-luer transfer attachment (2218 B, Tulip Medical Products, San Diego, CA). The fat is emulsified by forcing it between the two syringes 20 times, and the 2.4-mm transfer attachment is then replaced with a 1.4-mm attachment (2034A, Tulip Medical Products). This fat was transferred 20 times, and then the syringe was attached to a 1.2-mm luer-to-luer transfer attachment (2034 B, Tulip Medical Products). The fat was then emulsified 20 times through a smaller 1.2-mm attachment. The fat in the syringe was connected to a final adaptor, which contained a metal mesh filter, and the emulsified fat was pushed through the metal mesh filter adaptor into a final 10-mL syringe. Aliquots of nanofat were then prepared in 1-mL syringes for injection through a 25-gauge butterfly needle.

Voice evaluation

Video stroboscopy, voice assessments, and clinical evaluations were conducted before treatment and 3 months post injection. Voice Handicap Index-10 (VHI-10) was administered before and during follow-up visits. For acoustic analysis, patients were recorded in a quiet room, repeating the "How hard did he hit him" from the Consensus Auditory-Perceptual Evaluation of Voice (CAPE-V). This voice token was done five times within a ten-second interval. The recordings were made with a microphone positioned six inches from the mouth in a room where the ambient noise was kept below 55 dB. The recordings were analyzed using the cepstral peak prominence method (ADSV Software for Analyzing Dysphonia in Speech and

Voice, Version J3.42; Pentax Medical Computer Speech Laboratory, Montvale, NJ). The Cepstral Spectral Index of Dysphonia (CSID) scores were calculated. The CSID index for the CAPE-V sentences was used as the acoustic analysis result.[22–24] Subjective perceptual voice ratings were obtained by two independent blinded experts using the GRBAS scale to perform subjective assessments of voice samples before and after treatment. The acoustic sample used is the CAPE-V sentence "How hard did he hit him?" repeated for 10 seconds.

Stroboscopy analysis

Stroboscopic evaluations were conducted before and after treatment. Two expert laryngologists, blinded to the treatment arms, judged the vocal fold closure of the phonation tokens. The raters were asked to rate closure as complete or incomplete based on the stroboscopy token of modal phonation using the chest register.

Statistics

Vocal fold closure ratings on stroboscopy, CSID scores, GRBAS scale ratings, and VHI-10 scores were compared before and after the treatment (two-tailed paired *t* tests and Mann-Whitney *U*). Pretreatment and post treatment data were compared.

RESULTS

A total of 18 patients (12 experimental, six controls) with vocal atrophy underwent fat injection laryngoplasty from August 2021 to July 2023. The study included three women and 15 men. The median patient age was 72 years (SD = 9 years). The youngest was 42 years old, and the oldest was 81. An average of 0.6 mL fat was injected into each vocal fold. The injection volume was the same in the experimental and control groups. All patients tolerated the injections and were discharged on the day of surgery.

This report describes the findings with a median follow-up of 3 months after the surgery.

Table 1 shows the pretreatment and post treatment VHI and CSID scores. All patients returned for follow-up with a median follow-up time of 3 months. The median VHI score for all patients who underwent fat injection laryngoplasty decreased from 22 to 17. The median CSID score decreased from 27.5 to 20 in all patients who underwent fat injection laryngoplasty. The VHI improved after the intervention ($P < 0.05$), and the CSID improved but was not statistically significant.

Tables 2 and 3 present the control and experimental groups' pretreatment and post treatment VHI and CSID scores, respectively. VHI scores for patients undergoing nanofat injection decreased from 19 to 15.5 after the intervention ($P < 0.05$, paired two-tailed *t* test), whereas VHI scores for microfat patients decreased from 22 to 17 ($P > 0.05$, paired two-tailed *t* test). While VHI was noted to be significantly improved within the nanofat subgroup after treatment, the change in VHI was not significantly greater in the microfat group compared to that experienced by the nanofat group ($P > 0.05$, Mann-Whitney *U*). The CSID for nanofat patients decreased from 37 to 26.5 3 months after surgery, and the scores for microfat patients decreased from 20 to 17.5. The post treatment CSID was not significantly improved in either subgroup ($P > 0.05$, paired two-tailed *t* test), and the CSID did not change more after nanofat compared with after microfat ($P > 0.05$, Mann-Whitney *U*).

Stroboscopy demonstrated improved closure in both groups. There was incomplete closure in 12 out of the 18 patients before surgery, and incomplete closure was

TABLE 1.
All Study Patients' Preoperative and Postoperative VHI and CSID Scores (*n* = 18)

	Pretreatment Score	3-mo Post treatment Score
VHI median (all)	22	17*
VHI SD (all)	7.8	5.5
CSID median (all)	27.5	20
CSID SD (all)	18.5	15.7

* = $P < 0.05$ (paired *t* test).
SD, standard deviation.
VHI improved significantly for all patients at follow-up ($P < 0.05$). Average CSID improved but was not statistically significant.

TABLE 2.
Preoperative and Postoperative VHI Scores by Subgroup

	Pretreatment score	3-mo post treatment score
VHI median (microfat)	22.0	17.0
VHI SD (microfat)	6.5	5.9
VHI median (nanofat)	19.0	15.5*
VHI SD (nanofat)	10.7	4.7

* = $P < 0.05$ (paired *t* test).
The average VHI score decreased (improved) significantly for patients receiving nanofat ($P < 0.05$). VHI improved but was not statistically significant for the microfat group.

TABLE 3.
Preoperative and Postoperative CSID Scores by Subgroup

	Pretreatment score	3-mo post treatment score
CSID median (microfat)	20.0	17.5
CSID SD (microfat)	15.2	15.1
CSID median (nanofat)	37.0	26.5
CSID SD (nanofat)	24.0	18.0

* = $P < 0.05$ (paired *t* test).
The average CSID score decreased (improved) more for patients receiving nanofat than those receiving microfat. Neither change was statistically significant ($P > 0.05$).

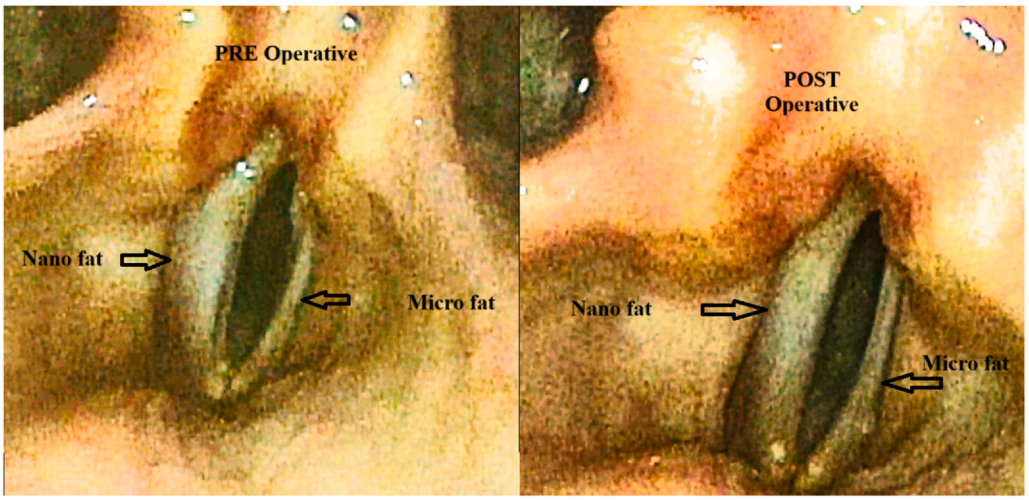

FIGURE 1. Video print of preoperative and postoperative glottis closure in the most open phase of the glottal cycle in a patient before (left) and after nanofat injection (right). Stroboscopy at 3 months demonstrates complete glottis closure. Nanofat was injected into the right vocal fold (arrow). Microfat was injected into the left vocal fold (arrow). Note the fuller right vocal fold (nanofat) at the postoperative photo compared with the left (microfat) side.

TABLE 4.
Judges Blinded to Treatment and Timing Rating of Complete Closure on the Stroboscopy Sample

	Complete closure before treatment	Complete closure 3 months after treatment
Microfat Patients $N = 6$	2/6 (33%)	5/6 (83%)
Nanofat Patients $N = 12$	4/12 (33%)	10/12 (83%)
All patients $N = 18$	6/18 (33%)	15/18 (83%)

Closure rates overall improved from 33% preoperatively to 83% postoperatively. There was no difference in the closure rates between the nanofat (n = 12) and microfat groups (n = 6) in the ability to achieve complete closure.

reduced to three patients postsurgery. There was no difference in the closure rates between the control and experimental arms. Figure 1 shows the glottis closure for a nanofat patient before injection and at the 3-month follow-up visit. It showed improved closure on both sides, with the right receiving nanofat and the left receiving microfat. Table 4 shows the two groups' closure rates before and after treatment.

Table 5 shows the patients' average GRBAS scores before and after treatment. Expert raters noted a significant improvement in dysphonia in the nanofat group but not in the microfat group. There were significant reductions in the severity of the overall grade of voice as well as levels of roughness, asthenia, breathiness, and strain ($P < 0.05$) in the nanofat group, but not in the microfat group. The change from baseline was statistically equivalent for all GRBAS scores when the microfat and nanofat subgroups were compared between groups ($P > 0.05$, Mann-Whitney U).

DISCUSSION

Nanofat injections may be an alternative to microfat injections in injection laryngoplasty. This study aimed to

TABLE 5.
Preoperative and Postoperative GRBAS Dysphonia Scores by Subgroup

	Pretreatment score (Nanofat)	3-mo post treatment score (Nanofat)	Pretreatment score (Microfat)	3-mo post treatment score (Microfat)
Overall grade	1.8	1.2*	1.8	1.6
Roughness	1.4	1.0*	1.3	1.3
Breathiness	1.2	0.5*	1.3	0.7
Asthenia	1.0	0.4*	1.2	0.8
Strain	1.8	1.0*	1.7	1.2

*=$P < 0.05$ (paired t test).
Expert raters noted statistically significant improvement in GRBAS scores for nanofat patients ($P < 0.05$). *Dysphonia scores did not change significantly for microfat patients. *denotes statistical significance.*

determine whether fat prepared as nanofat can be used as a safe bulking agent in patients with vocal atrophy. We were interested in considering nanofat because of its potential for better fat-derived stem cell retention than microfat. Tissue regeneration using stem cells, growth factors, and extracellular matrices may play a role in vocal atrophy, and its potential has been demonstrated. Fat is a well-known stem cell reservoir used for injection laryngoplasty since 1992.[25] This technique uses microfat—minute adipose tissue fragments to bolster the vocal fold and improve glottis closure. Despite its effectiveness in improving voice quality, the variability in fat retention has spurred the pursuit of alternative methods.[26] In the facial plastic surgery literature, refinements in how fat is prepared have promoted the use of nanofat.[27] Although microfat may be helpful for volume, nanofat is thought to be better at improving texture. This effect is thought to be owing to the better retention of fat stem cells.[18]

Nanofat is a novel material for vocal fold lipoinjection.[18,21] Its reduced volumetric capacity is offset by its potential for enhanced graft survival, suggesting a more consistent volume retention post procedure. In this study, we did not observe a significant volume loss on the sides with nanofat compared to that of microfat. The two groups had no significant differences in the objective voice results. Both groups reported subjective improvements in their voice handicap indices. Only nanofat patients showed significant changes in the VHI. Given its equivalence to microfat, nanofat may be a viable alternative.

There are other advantages to using nanofat. Injection of nanofat involves fine needles, of which smaller needles reduce vocal fold trauma and complications. A small needle, mimicking the size of the injection needle currently used for injection laryngoplasty, may also allow the procedure to be performed in an office setting.

The limitations of this study include the small number of patients treated and the short-term follow-up of the patients. This paper is a preliminary study to see if nanofat is equivalent to microfat. We recruited only 18 patients from among the many patients with vocal atrophy over 2 years. Many patients opted for standard care with microfat and chose not to undergo randomization, and hence, were excluded from this study. It is important to interpret these results with caution. The absence of statistical significance for patients with microfat and the nonuniform improvement across all metrics may be owing to the small number of patients in the microfat group. Caution should be exercised when interpreting these results. Given the variability of results from fat injection over time, long-term data using nanofat will also need to be reported. However, nanofat may be considered an alternative to standard microfat for injection laryngoplasty in patients with vocal atrophy. Nanofat for injection laryngoplasty requires further investigations.

CONCLUSION

Nanofat may be an alternative to microfat for patients treated with injection laryngoplasty. This preliminary study indicates that patients receiving nanofat injections exhibit subjective and objective improvements comparable to those receiving microfat during the three-month follow-up period. Stroboscopy showed good closure of the glottal gap after nanofat injection laryngoplasty. Nanofat injection into the larynx may be a new method for injection laryngoplasty.

Declaration of Competing Interest

All authors have no conflicts of interest to declare.

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