Nanofat: A novel therapeutic approach to autologous fat transfer in glottic insufficiency

Background

Glottic insufficiency is caused by paralysis, atrophy and scarring of the vocal folds and is one of the leading causes of dysphonia, significantly affecting patients' quality of life (1). Several techniques have been described to treat glottal insufficiency and may involve open surgery or injection laryngoplasty. While open surgery can be effective, it has limitations and greater risk of complication. Injectable therapies represent a more conservative, but effective therapy. Laryngeal augmentation aims to close the glottic gap by increasing the volume of the affected vocal fold to medialise the vibratory edge, where it can participate in phonation(3). When scarring is present, anti-fibrotic therapies such as steroid injection may also be trialled in order to soften scar, however multiple injections are required, and scarring may recur.

A variety of materials have been described for injection laryngoplasty, such as collagen, hyaluronic acid, carboxymethylcellulose and autologous fat in order to increase the volume of the paralysed cord and medialise it. The use of autologous fat has intuitive appeal, as the substance has highly desirable vibratory/rheological properties, is bio-compatible, readily available, and repeatable(4). Additionally, fat tissue is naturally rich in anti-fibrotic cell-signalling molecules and cells, including stem cells, and therefore can theoretically simultaneously provide volume while reducing vocal fold scarring(5, 6). Perhaps the most desirable property, borne out in a few studies, is its potential longevity. In theory, and in a few small studies, autologous fat injection has been shown to last for many years, even permanently, surviving essentially as a vascularised free graft. Unfortunately, such consistent in-vivo longevity, despite various described harvesting and processing techniques, remains elusive(7).

Lipotransfer, or fat grafting, is the technique of taking autologous fat from another part of the body and implanting in another. The utility of fat grafting has been extensively described in aesthetic and reconstructive literature. Autologous fat injection for vocal cord paralysis has been described to manage glottic insufficiency, and its effects have been found to be sustainable in some studies (8, 9). Beyond fat's favourable properties as a filling agent, autologous fat also contains important anti-fibrotic and immunomodulatory properties conferred by its stroma. This so-called stromal vascular fraction (SVF) is the non-adipocyte, supportive component of harvested fat and contains a complex population of fibroblasts, macrophages, immune regulatory cells, and a high density of adipocyte derived stem cells (ADSC), a type of mesenchymal stem cell (MSC)(5). Additionally, the non-cellular component of SVF contains a complex milieu of regulatory factors that modify extracellular matrix (ECM) production and cellular behaviour(6).

There has been recent interest in supplementing standard fat grafting techniques with SVF to improve graft survival (10, 11), with the rationale that the complex population of progenitor cells and supportive factors may help maintain the grafted adipocytes and improve long-term graft retention. Previously the harvesting and refinement of MSCs has required expensive specialist equipment and extended processing times meaning that patients would have to undergo harvesting and re-implantation as two separate procedures. Now, numerous studies have described a novel technique, to harvest fat and derive SVF intraoperatively, allowing for same-day reimplantation(6).

First described by Tonnard et al. the production of this so-called nanofat, involves mechanically emulsifying and filtering harvested fat. The technique does not require the use of a surgical laboratory, proteolytic enzyme reagents, or specialised equipment (closed

automated system, i.e. Celution® system). In their study, Tonnard et al demonstrated that the nanofat samples contained no viable adipocytes, but still maintained a population of mesenchymal stem cells alongside other crucial SVF components. Given this finding, the authors suggest that nanofat has a very limited role in building up significant fat volume. Rather, nanofat injection can be used to supplement microfat grafting through stem cell activity. Several studies in the plastic and reconstructive literature have demonstrated the utility of adding nanofat to graft material in order to improve the longevity of fat grafts(6). To date, there is no study evaluating the use of nanofat in volume augmentation of vocal folds, such as in cases of vocal fold paralysis, or atrophy due to other causes. In the case of scarring, MSCs have been shown to reduce the proliferation of scar fibroblasts and impede myofibroblast differentiation, while at the same time promoting extracellular matrix production with high densities of hyaluronic acid and reduced collagen-1. Nanofat has had favourable results in its use for skin scars in the reconstructive plastics literature (6, 12) and more recently has shown promising results in improving vocal fold pliability in patients suffering from vocal fold scar(13).

Aims:

The aim of this project is to explore the utility of nanofat in the treatment of glottic insufficiency in two ways:

1. We aim to determine whether supplementation of traditional fat grafting with nanofat in patients with unilateral vocal cord paralysis can prolong graft survival by comparing patients (traditional fat grafting versus fat graft with nanofat) based on:
    a. Stroboscopic measures of closure and vibration
    b. Acoustic and aerodynamic measures
    c. Patient reported outcome measures (PROMs): VoiSS & perceived overall voice impairment on VAS
2. We aim to determine whether nanofat alone is effective in reducing vocal fold scarring and restoring natural vocal fold pliability by comparing patients (steroid injection versus nanofat injection) based on:
    a. Stroboscopic measures
    b. Acoustic and aerodynamic measures
    c. Patient reported outcome measures (PROMs).

Nanofat for unilateral Vocal Cord Paralysis

Hypothesis

We hypothesize that the use of nanofat supplementation of traditional autologous fat grafting in vocal fold augmentation leads to greater autograft longevity compared with traditional fat grafting techniques. We hypothesise that this longevity will be seen in the slower rate of deterioration of various stroboscopic, phono-acoustic and aerodynamic measures of glottic closure/function, compared with traditional fat transfer techniques.

Methods

Nanofat will be harvested using a technique similar to that described by Tonnard et al. A device produced by Samson Medical Technologies known as an ‘Adinizer’ will be used to process the nanofat.

We expect to recruit a total of 20 patients. Patients are recruited into one of two groups. Group 1 (n=10) will receive vocal fold injection with traditional volume injection augmentation using autologous fat. Group 2 (n=10) will receive a combination of autologous fat injection and nanofat. Each patient will receive a measured volume of fat injection, adjusted according to individualised clinical need & surgeon judgement. In addition, each patient will have a different volume of vocal fold loss from paralysis, which may be related to individual patient variation in anatomy and duration of paralysis. In the nanofat group, patients will receive standard fat injection to medialise the paralysed cord to the midline. They will also receive an additional 50% (of total microfat injected volume) of nanofat.

All patients will have a baseline analysis pre-injection. This will include stroboscopic measures, phono-acoustic and aerodynamic measures, and voice questionnaire. Post injection, assessments will be carried out at 1 week, 3 months, 6 months and 12 months. Laryngeal assessments will be performed by videolaryngostroboscopy to visualise and video-document the degree of glottic gap at rest and with phonation, and the nature of the mucosal wave on the paralysed/augmented side. Acoustic (including harmonic:noise ratio, perturbation, fundamental frequency and intensity) and aerodynamic measures (phonation threshold pressure, mean subglottic pressure, s:z ratio) will be undertaken. The VOISS patient reported outcome measure will be administered to assess perceived voice-related disability at each assessment.
Patients will be consented to participate in this study.
Patients will be recruited from the laryngology clinic at Monash Health. Patients presenting with vocal fold scarring will be approached by the primary investigator (PP) and offered to participate in the study. A 'patient information and consent form' (PICF) will be used to aid the consent process. Patients will have the opportunity to ask any questions and consider their participation in the study, up until the clinic-visit prior to first surgery. Patients may withdraw their consent at any time using the PICF. No vulnerable groups will be intentionally targeted as part of our recruitment strategy. Patients will be recruited consecutively, and randomised on a sequential basis into either group 1 or 2. Inclusion criteria will require participants to be competent adults with unilateral vocal cord paralysis. Exclusion criteria will include: evidence of other vocal fold abnormalities (granuloma, polyps, sulcus or other pathology affecting vocal fold vibration), previous history of glottic cancer, previous laser resection of the vocal fold, prior radiotherapy.
Mechanism (if known) of vocal cord paralysis and duration of paralysis will be recorded for each patient. Patients will be age and sex-matched between groups. All injections will be performed by a single operator. Patients will be blinded to their group allocation. The assessors involved in post-injection radiological and stroboscopic analyses will be blinded to the group allocations.

Results and Analysis:
Outcomes of interest will include rate of change of all the above parameters, in the 'Nanofat' group compared with that in the 'control' group. Inter and intra-group comparison will be made between pre and post-injection voice questionnaire scores and stroboscopic and aerodynamic parameters.

Nanofat for vocal fold scar

Hypothesis

Methods
Nanofat will harvested and processed as described above
We aim to recruit a total of 20 patients with symptomatic scar/fibrovascular change of one or both cords. Patients will then be randomised into one of two groups. Group 1 (n=10) will undergo traditional, submucosal steroid injection (dexamethasone 4mg/ml) into the scarred portions of one or both cords as per standard clinical practice. Group 2 (n=10) will undergo superficial submucosal injection of nanofat into one or both cords, depending on clinical indication. No unprocessed 'microfat' will be utilised. As per standard clinical practice for steroid injection in vocal fold scar, each group will undergo 3 serial treatments of either dexamethasone or Nanofat. Each treatment will occur 1 month apart. Each patient will receive a measured volume of steroid or Nanofat, adjusted according to individualised clinical need & surgeon judgement at the time of procedure.
All patients will have a baseline analysis pre-injection. Patient demographics and scar aetiology will be noted. A voice sample will be recorded for perceptual and acoustic voice evaluation purposes and a stroboscopic evaluation for disease morphology, vocal fold vibration impairment and fold mucosal abnormalities. Aerodynamic assessment will also be undertaken for data capture of phonation threshold pressure, mean subglottic pressure and

s:z ratio. The 'VOISS' patient reported outcome measure will be administered to assess perceived voice-related disability at each assessment. The same assessments will be applied 4-6 weeks and 10-12 weeks after the second and third injections respectively.
Patients will be recruited from the laryngology clinic at Monash Health. Patients presenting with vocal fold scarring will be approached by the primary investigator (PP) and offered to participate in the study. A 'patient information and consent form' (PICF) will be used to aid the consent process. Patients will have the opportunity to ask any questions and consider their participation in the study, up until the clinic-visit prior to first surgery. Patients may withdraw their consent at any time using the PICF. No vulnerable groups will be intentionally targeted as part of our recruitment strategy. Patients will be recruited consecutively, and randomised on a sequential basis into either group 1 or 2. Inclusion criteria will require participants to be competent adults with vocal fold scar. Exclusion criteria will include: evidence of other vocal fold abnormalities (granuloma, polyps, sulcus or other pathology affecting vocal fold vibration), previous history of glottic cancer, previous laser resection of the vocal fold, prior radiotherapy, as well as patients not fit for surgery due to anaesthetic risk.
Aetiology of vocal fold scar and duration of scar will be recorded for each patient. Patients will be age and sex-matched between groups. All injections will be performed by a single operator. Patients will be blinded to their group allocation. The assessors involved in post-injection stroboscopic analyses will be blinded to the group allocations.

Postoperatively all patients will receive standard instructions for voice rest for 24 hours. All patients will receive standard voice therapy follow-up post-injection.

Results and Analysis:
Outcomes of interest will include rate of change of all the above parameters, in the 'Nanofat' group compared with that in the 'control' group. Inter and intra-group comparison will be made between pre and post-injection voice questionnaire scores and stroboscopic and aerodynamic parameters.

Research Assistant / Research Nurse / Speech Therapy

We plan to employ a research assistant projected to be employed for 0.5 FTE. They will be responsible for following-up consented patients and administering voice questionnaires perioperatively. They will organise and collate all consent forms, vocal assessment outcomes for data entry, and MRI bookings. They will be responsible for contacting participants for routine post-procedure follow-up. In addition, a part-time speech therapist will be involved in this study to provide standardised post-procedure speech therapy sessions to all participants in this study.

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