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# Treatment of glottis insufficiency with vocal fold injection

## Zdravljenje glotisne insuficience z injiciranjem v glasilke

Nejc Krištofelc,[1] Irena Hočevar Boltežar[1,2]

[1] Faculty of medicine, University of Ljubljana, Ljubljana, Slovenia
[2] Department of Otorhinolaryngology and Cervicofacial Surgery, University Medical Centre Ljubljana, Ljubljana, Slovenia

**Correspondence/ Korespondenca:**
Irena Hočevar Boltežar, e: irena.hocevar@kclj.si

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## Abstract

**Background:** Sulcus, scar, paresis and atrophy of the vocal fold are the causes of glottis insufficiency, and disordered propagation of the mucosal wave over the vocal fold. Thus, these are indications for vocal fold injection augmentation. Patients usually complain about dysphonia, though problems with swallowing may also be present.

**Methods:** In order to achieve vocal fold augmentation, material is injected deep into the thyroarytenoid muscle thereby increasing its volume and reducing glottic insufficiency. Different temporary and long lasting materials can be used depending on patient's symptoms and prognosis. The procedure can be performed by direct microlaryngoscopy under general anaesthesia. Due to numerous advantages and development of transcutaneous approaches, outpatient-based injection technique is becoming more popular. Tissue engineering together with in-depth knowledge of the complex composition of mucosal lamina propria on the vocal folds, and changes during scarring process in it has a big potential for preventing and treating vocal fold scar that results in incomplete vocal fold closure during vocal fold adduction.

**Own experience:** Since 2006, patients with glottis insufficiency have been treated by autologous fat injection to one or both vocal folds at the University Department of Otorhinolaryngology and Cervicofacial Surgery, University Medical Centre Ljubljana. In most patients, the reason for incomplete vocal fold closure is the impaired mobility of the vocal fold, the main symptom being hoarseness and frequent aspiration during drinking. The treatment was successful in the vast majority of patients; their voice quality improved and their swallowing problems were usually reduced or disappeared. Only a small number of patients needed reinjection in a few years' time due to deteriorated contact between the vocal folds. The subjective improvement is also confirmed by the results of acoustic voice analysis before and after the procedure.

## Izvleček

**Izhodišče:** Za injiciranje različnih materialov v glasilko se odločimo v primeru sulkusa, brazgotine, pareze in atrofije glasilke. Ta stanja povzročajo nepopolno zaporo glotisa in vplivajo na normalno širjenje sluznicnega vala glasilke. Glavna bolnikova težava je običajno disfonija, lahko pa so prisotne tudi težave pri požiranju.

**Metode:** Za okrepitev glasilke snov vbrizgamo globoko v tiroaritenoidno mišico, s tem povečamo njeno prostornino ter zmanjšamo glotisno insuficienco. Glede na izraženost bolnikovih težav in napoved izida uporabljamo različne začasne in dolgo obstojne materiale. Poseg se lahko opravi v splošni anesteziji z direktno mikrolaringoskopijo, v zadnjem času pa se zaradi številnih prednosti in razvijanja transkutanih pristopov vse bolj uveljavlja tudi ambulantno injiciranje v lokalni anesteziji. Velik potencial ima področje tkivnega inženirstva, ki razvija materiale za preprečevanje in zdravljenje brazgotine glasilke, kar je vzrok za nepopolni stik med glasilkama pri addukciji.

**Lastne izkušnje:** Od leta 2006 dalje zdravimo bolnike z glotisno insuficienco z vbrizganjem lastne maščobe v eno ali obe glasilki tudi na Kliniki za ORL in CFK UKC Ljubljana. Pri večini bolnikov je vzrok za nepopoln stik med glasilkama motena gibljivost glasilke, vodilni težavi sta hripavost in manj pogosto zaletavanje pri pitju. Zdravljenje je bilo uspešno pri veliki večini, glas se je izboljšal, težave pri požiranju so se praviloma zmanjšale ali izginile. Le pri manjšem številu bolnikov je bilo čez nekaj let potrebno injiciranje ponoviti zaradi poslabšanja stika med glasilkama. Subjektivno izboljšanje potrjujejo tudi rezultati akustične analize glasu pred in po posegu.

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## 1 Causes of glottis insufficiency

The main functions of the throat are breathing, airway protection during swallowing, and voice formation. In the case that altered vocal fold mobility or disease related changes prevent complete contact of the vocal folds during phonation or swallowing, or interfere with the travel of the mucosal wave from the lower to the upper surface of the vocal fold during oscillation, vocal disorders and/or aspiration during swallowing may occur. In the case of sulcus, scar, paresis and vocal fold atrophy, we decide to strengthen the vocal fold by injecting various materials into it.

**Sulcus vocalis** is an epithelial invagination along the free edge of the vocal fold. It can be limited to the surface layer of the lamina propria or extend deeper into the vocal fold layer. Ford distinguishes three types of sulcus according to the depth of change. Type-I sulcus (physiologic sulcus) is limited to the superficial layer of the lamina propria. Type-IIa Sulcus (sulcus vergeture) indicates the absence of a functional superficial layer of lamina propria and is most commonly adherent to the vocal ligament (intermediate and lower layer of lamina propria). Type-IIb sulcus (true sulcus) extends into the vocal ligament or even into the thyroarytenoid muscle. Opinions on the causes of sulcus are divided. Improper and excessive use of voice is mentioned as the most common cause, but it can also be due to damage to the vocal fold, infection or laryngopharyngeal reflux. It is also possible that it is a congenital anomaly caused by abnormalities in the development of the fourth and sixth bronchial arches. Sulcus may be due to the formation and subsequent rupture of an epidermoid cyst on the vocal fold. Sulcus is always accompanied by scarring, as fibrosis and epithelial growth on the underlying layer develops in the area of its bottom (1,2).

**A vocal fold scar** is caused by a vocal fold injury. This occurs due to chemical and thermal factors (smoking, laryngopharyngeal reflux, inhalation burn of the larynx, laser removal of benign lesions), penetrating or blunt larynx injuries, long-term intubation, after irradiation of the larynx and surgical removal of benign and malignant lesions (injuries) of vocal folds (Reinke's edema, polyps, nodules, papillomas, throat cancer). Similar to sulcus vocalis, the scar is most often the result of voice abuse. Damage to the vocal fold disrupts the balance between the components of the extracellular matrix of the lamina propria. Type III collagen begins to be replaced by Type I collagen,

the amount of elastin and hyaluronic acid decreases, and the proportion of fibronectin increases. The amount of the decorin proteoglycan is also reduced, resulting in disorganized deposition of collagen fibres. All this leads to scar formation, which during the phonation oscillation of the vocal folds prevent the complete closure of the glottis and the normal propagation of the mucosal wave over the vocal folds, as they destroy the so-called "body-cover" model of the vocal fold. In the latter, the epithelium, basement membrane, and superficial layer of the lamina propria act as a common functional unit (vocal fold cover) and move wave-like over the base (body of the vocal fold) formed by the lower layer of lamina propria, which grows tightly on the thyroarytenoid muscle. If a scar forms, adhesions appear between the two units, impeding vocal fold oscillation. When scarring occurs due to vocal fold excision or vocal fold irradiation during cancer treatment, glottis insufficiency is often severe and the rest of the vocal fold is severely scarred (3,4).

**Vocal fold paresis or paralysis** is the result of a defect at the level of the brainstem, along the course of the 10th cranial nerve (n. vagus) or its branch of the recurrent laryngeal nerve (RLN). The most common cause remains iatrogenic injury to the innervation of the vocal fold, which occurs during various medical procedures, such as thyroidectomy, parathyroidectomy, anterior approach to cervical spine surgery, skull base procedures, esophageal surgery, thymectomy, mediastinoscopy, neck dissection, carotid endarterectomy, thoracic aortic aneurysm surgery, coronary artery surgery, open Botallo's duct ligation, pneumonectomy, and lobectomy. Various benign and especially malignant tumors along RLN are also a common cause. The most common are metastases of the primary lung tumor in the mediastinal lymph nodes, and less commonly the cause is thyroid and esophageal tumors and paragangliomas. RLN can also be affected by nonmalignant diseases such as mediastinal lymphadenopathy in tuberculosis and sarcoidosis, and aortic and right subclavian artery aneurysms. The left vocal fold is more often affected than the right (60:40 ratio), because due to the longer and deeper course in the mediastinum, the left RLN is more vulnerable to various diseases and injuries during surgery. Neurological causes of vocal fold paresis include stroke, Arnold-Chiari malformation, multiple sclerosis, Guillain-Barré syndrome, amyotrophic lateral sclerosis, and Parkinson's disease. Irradiation due to malignant diseases in the neck and mediastinum leads to fibrosis of the irradiated tissue and can be manifested by paresis of the vocal folds only several decades after irradiation. Some drugs and toxins, such as the vinca and cisplatin alkaloids, are neurotoxic, and the problems usually disappear after a dose reduction or cessation of treatment. Many times, the cause of vocal fold paresis is not discovered. This is called idiopathic paresis, which could be due to inflammatory neuropathy in the context of Lyme borreliosis or infection with herpes simplex and Ebstein-Barr viruses. Idiopathic paresis should always be diagnosed after excluding other causes (5,6).

**Vocal fold atrophy** can be the result of vocal fold paresis or paralysis, various muscle diseases, often accompanied by sulcus and scar on the vocal fold. It also occurs due to age-related changes in the larynx, which is called *presbylarynx* in Latin. Age-related atrophy of the vocal folds is due to the loss of axons and reduced myelination of the laryngeal nerves, thinning of the thyroaritenoid muscle with collagen infiltration, fatty degeneration and a decrease in the proportion of hyaluronic acid in the lamina propria, which changes the viscoelastic and vibrational properties of the vocal folds. The population is aging, so more and more seniors have voice problems. These may be due to a variety of causes that must be ruled out before diagnosing age-related changes in the throat (5,7).

## 2 Clinical picture of glottis insufficiency

Impaired mobility of the vocal fold due to neurogenic impairment (paresis, paralysis of the vocal cords) or atrophy causes glottis insufficiency or incomplete contact between the vocal folds during phonation. In addition, structural changes in the vocal folds (sulcus, scar) also reduce the mobility of individual layers of the mucosa between each other and thus affect the normal propagation of the mucosal wave over the vocal fold during its oscillation. Patients therefore have a hoarse and weak voice, they get tired quickly when speaking, they run out of air, the pitch of the voice is often not appropriate, and due to the compensatory tension of the laryngeal muscles it is usually too high. As the larynx plays an important role in the protection of the lower respiratory tract during swallowing, aspiration during swallowing may also occur, especially when drinking clear liquids (8).

## 3 Treatment of glottis insufficiency

Treatment is aimed at improving voice quality, preventing dysphagia and aspiration. It must be causal and tailored to each patient individually. In doing so, we take into account the expression of the patient's problems, his or her own desires and his or her profession. Possible treatments include voice therapy, vocal fold medialization by injection or thyroplasty, arytenoid adduction, and recently laryngeal reinnervation, functional electrical stimulation, and even gene therapy have become increasingly popular in the treatment of vocal fold paresis (8).

A big challenge is treating sulcus and scarring. In addition to medialization, we can also use a surgical or laser excision of the lesion, which is often combined with the injection or implantation of various substances into the lamina propria. The scar together with the epithelium is separated from the vocal ligament, and materials with similar viscoelastic properties as lamina propria are then injected or implanted into the resulting submucosal pocket, which does not affect its biomechanical properties. These are autologous fat, fascia and hyaluronic acid. The use of an anxiolytic laser, buccal mucosa transplantation and rapidly evolving tissue engineering are also promising (4).

### 3.1 Medialization of the vocal fold by injection

Injection of various materials into the vocal fold has a history of more than 100 years, as the German otorhinolaryngologist Wilhelm Brünings was the first to perform vocal fold medialization in 1911 when he injected paraffin into it. However, the procedure has flourished in the last three decades, mainly due to the use of new materials and the development of endoscopic technology and various approaches.

The injection of the substance into the vocal fold enables medialization, in which the free edge of the vocal cord is brought closer to the median plane of the glottis, thus enabling contact with the other vocal fold during phonation or swallowing. We decide to use injection if the gap between the vocal folds is less than 3 mm, while the best results are recorded at a distance of less than 1 mm. In larger gaps, thyroplasty is more successful, in which the surgeon cuts out a portion of the thyroid cartilage and moves it between the cartilaginous skeleton of the larynx and the immobile vocal fold, or uses an implant to push it into the inside of the throat through a cut window in the thyroid cartilage. This achieves a better position and tension of the vocal fold. Thyroplasty with or without arytenoid adduction gives better results in posterior glottis insufficiency (9,10).

The substance is injected deep into the thyroarytenoid muscle as lateral as possible from the vocal process of the pyramid-shaped cartilage, and if neces-

sary, into the anterior part of the vocal fold, thus increasing its mass. Since upon the patient's awakening from anesthesia, coughing and speaking immediately after the procedure, as a rule, some injected substance leaks out of the vocal fold, and some is also resorbed, we always make at least a 30% hypercorrection. We must be aware that by injecting the substance into the thyroarytenoid muscle, we only improve the position of the vocal fold and thus reduce glottis insufficiency, but we do not eliminate the disturbed transmission of the mucosal wave due to structural irregularities on the vocal fold surface at the scar and sulcus. For this purpose, a substance with a similar viscoelasticity as the vocal fold mucosa (fat, hyaluronic acid) can be injected into the surface layer of the lamina propria (Reinke's space). This restores the propagation of the mucosal wave, and at the same time medializes the vocal fold. For the time being, experts rarely decide to inject substances into Reinke's space (4,5,11).

## 4 Temporary or permanent medialization of the vocal fold

Medialization of the vocal fold by injecting various substances into it can be temporary or permanent.

**Temporary medialization** is considered in vocal fold paresis, when the prognosis of the outcome of spontaneous regeneration of the vocal fold innervation is not yet clear. Reinnervation can occur in the first 6-12 months after the onset of the damage, during which time the injection of short-lived substances improves the quality of the voice and alleviates swallowing problems. We choose this especially for people who need a good voice function to perform their profession (teachers, professional speakers, lawyers, call centre workers, priests and actors) and for people with severe dysphagia and therefore the risk of aspiration. Temporary injection is also chosen when it is not clear whether glottis insufficiency, which causes dysphonia, is the main cause of communication problems, or whether these are the result of another concomitant speech disorder, e.g. dysarthria. Before possible subsequent permanent medialization, the patient is also shown to what extent the quality of his or her voice can be improved by injection. Saline solution, bovine gelatin, carboxymethylcellulose, collagen and hyaluronic acid are used for temporary medialization. These substances disappear from the vocal fold in a few weeks or months (5).

If spontaneous regeneration of the innervation of the vocal fold with paresis does not occur or the possibility of restoring of its function at the time of diagnosing paresis is small, we opt for **permanent medialization**. Clinical experience shows that vocal fold mobility in paresis due to the growth of malignant tumors in the RLN or complete rupture of the RLN is rarely improved. In determining the prognosis of the outcome of the improvement of neurogenic impairment, laryngeal electromyography can be used, which shows the degree of denervation or reinnervation of the vocal fold. By injecting permanent substances into the vocal fold, we can reduce glottis insufficiency in the sulcus and scar of the vocal fold quite successfully, and with it we can also make small corrections in the medialization of the vocal fold after thyroplasty, which we'd performed before that (so-called fine tuning). In the past, paraffin, glycerin and silicone were injected into the vocal folds, but they were abandoned due to poor long-term results or even side effects. Today, autologous fat, autologous fascia (*fascia lata*), calcium hydroxyapatite and to a lesser extent Teflon are used to permanently strengthen the vocal folds (5,9,11).

## 5 Possible vocal fold injection approaches

Injection medialization of the vocal fold can be done by direct microlaryngoscopy under general anesthesia or as an

outpatient procedure with various transcervical, oral, and transnasal approaches under local anesthesia. It is technically more demanding in local anesthesia, but it has many advantages compared to the injection under general anesthesia. It is suitable for patients in whom general anesthesia is dangerous due to associated diseases. It enables clinical and stroboscopic assessment of voice quality during or immediately after injection and thus accurate dosing of the substance. The procedure under local anesthesia is also much faster and cheaper. Of course, good patient participation during the procedure is crucial for success. On the other hand, a procedure under general anesthesia with direct microlaryngoscopy allows a more accurate display of anatomical structures and therefore better control of the injection site and the distribution of the injected substance. It is possible to reassess the correct diagnosis, at the same time we can also perform additional procedures, such as acquisition of autologous fat or fascia (12,13).

There are three different transcervical approaches for vocal fold injection: cricothyroid, transthyroid, and thyrohyoid approaches. In all of them, we have to carefully palpate the anatomical structures of the larynx and determine the entry point of the needle. A local anesthetic is infiltrated into the skin above the injection site, and appropriate anesthesia of the nasal mucosa, pharynx and larynx should be provided. This is performed under the supervision of a flexible nasolaryngoscope or a rigid telelaryngoscope to show the vocal folds and paraglottic space. The best view of the throat is obtained if the patient is in a sitting or lying position with the neck slightly extended (the so-called "sniffing position"). Most commonly the cricothyroid approach is used. Here we enter the throat through the cricothyroid membrane just below the lower edge of the thyroid cartilage, then we gently push the needle upwards and laterally into the paraglottic space, where the substance is injected into the thyroarytenoid muscle. In the transthyroid approach, the needle is pushed perpendicular through the thyroid cartilage slightly lateral to the median line. We can also help ourselves by determining the upper edge of the vocal folds, which usually lies in the middle of the height of the thyroid cartilage in the line of the thyroid incision. The cartilage must be pierced in the middle between the upper edge of the vocal folds and the lower edge of the thyroid cartilage, and the needle is then directed laterally towards the paraglottic space. The substance is then injected into the vocal fold, and endoscopically observed to increase its volume and medialization. This approach is especially suitable for younger patients, as with age the thyroid cartilage becomes more and more calcified and therefore harder. In the thyrohyoid approach, the needle is inserted through the thyroid membrane just above the upper edge of the thyroid cartilage above the thyroid incision. The needle is pushed vertically down through the preepiglottis space when the needle is seen at the tip of the epiglottis, directed towards the vocal fold, and the substance is injected into it. Of all these three approaches, the thyrohyoid approach allows the most accurate injection, as the laryngoscope allows us to directly see the path of the needle (9,12,14).

In the oral approach, a special curved needle is inserted through the mouth and the substance is applied laterally from the vocal process. Successful surgery requires good anesthesia of the base of the tongue, soft palate, supraglottis and glottis, and appropriate patient anatomical features that allow a good view of the larynx. Otherwise, the injection is easier to perform with a transcervical or transnasal approach, in which a flexible needle is inserted through the fibrescope of the nasolaryngoendoscope (9,12,14).

Under general anesthesia, injection is performed by direct microlaryngoscopy. Access to the larynx and vocal folds is established with a rigid laryngoscope,

through which a needle is inserted. The injection site is exactly the same as for the mouth or nose approach (9,12,14).

## 6 Materials for vocal fold injection

Substances can be divided into two groups. In the first group are those that stay in the vocal fold for a short time. These are bovine gelatin, collagen, carboxymethylcellulose and hyaluronic acid. The second group includes substances that remain in the vocal fold for a longer time or forever. These are autologous fat, autologous fascia, calcium hydroxyapatite and Teflon. Some of these substances were originally developed as dermal fillers and later began to be used for injection into the vocal folds. Due to the lack of clinical trials on the efficacy and safety of their use for injection into the vocal folds, some of these substances are currently used in this field without an officially recognized indication. This is the so-called »off-label« use (5).

The ideal substance should be biocompatible, inert, easily accessible, easy to inject, resistant to resorption or migration outside the injection site, inexpensive, and as close as possible to the biomechanical and viscoelastic properties of the vocal fold. There is no ideal substance for injection, so it is important to know their advantages and disadvantages in order to choose the best option in each situation (15).

Among collagen preparations, the longest being used is bovine collagen (Zyplast®, Zyderm®), which remains in the vocal fold for 4-6 months. In a population, up to 3.5% of people may experience an allergic reaction, so skin testing is needed beforehand, which delays the treatment itself by a few weeks. Collagen derived from cadaveric acellular dermis (Cymetra®) does not cause an allergic reaction, but there is a risk of transmitting the infection. Therefore, gentamicin is used for its preparation. The use of this material is thus contraindicated in persons allergic to the mentioned antibiotic. According to some image analyses, cadaveric collagen is supposed to degrade only after 9 months or even later, but most experts believe that this happens after 2-3 months. Collagen derived from human dermis (Cosmoplast®, Cosmoderm®) is a relatively new material that is not currently used for laryngeal procedures. Compared to other collagen preparations, it has the advantage of not being associated with the risk of an allergic reaction and transmission of the infection. It is expected to degrade after 4-6 months, but further research is needed to determine the exact duration. Patients with systemic connective tissue diseases (rheumatoid arthritis, systemic lupus erythematosus, scleroderma) are at increased risk of an allergic reaction when using collagen preparations, so caution should be exercised (15).

**Hyaluronic acid** (Restylane®, Hylaform®) is a glycosaminoglycan of the extracellular matrix that is naturally present in many tissues of the human body, including the lamina propria of the vocal fold mucosa. It plays an important role in maintaining the complex composition and organization of the lamina propria and determines its viscoelastic properties. Because it is biocompatible, non-allergenic, has anti-adhesive properties and the ability to regenerate the tissues into which it is injected, it has been the subject of much research in recent years on the prevention and treatment of vocal fold scarring. In rabbit and dog models, injection of hyaluronic acid into the vocal fold immediately after injury has been shown to reduce the rate of fibrosis. It also gives positive results when injected into an already formed scar, where it restores the propagation of the mucosal wave and restores the composition of the lamina propria. Hyaluronic acid, together with its autologous fat, comes closest to the viscoelastic properties of the vocal fold, so it is also suitable for surface injection. Hyaluronic acid itself degrades in 3-5 days, and with tissue

engineering methods, its molecules can be cross-linked into a network structure. This makes hyaluronic acid more stable and slows its resorption from the vocal fold. It is therefore more durable than other temporary materials, and the clinical improvement of the voice after injection lasts 6-12 months. Due to the slower decomposition and the fact that it binds water, less hypercorrection is required during the procedure (6,15,16,17,18).

**Carboxymethylcellulose** (Radiesse Voice Gel®) improves voice quality well and is not associated with an increased risk of an allergic reaction. It degrades after 2-3 months, which is faster than collagen preparations and hyaluronic acid (15).

Substances from beef gelatin (Gelfoam®, Surgifoam®) are safe and remain in the vocal fold for 4-6 months. Due to their high viscosity, they need to be injected with a thicker needle, which reduces the accuracy of the procedure (9).

As a material, **autologous fat** has many beneficial properties. Because it is an autologous tissue, it is inert and completely safe to use and of course free. It has almost identical viscoelastic properties as the vocal fold mucosa, so it does not significantly affect the propagation of the mucosal wave during phonation and successfully improves voice quality due to glottis insufficiency. Immediately after the procedure, fat is resorbed to a greater extent than other materials, so more extensive hypercorrection is required, which makes patients experience dysphonia for a few weeks after the procedure. Hypercorrection can become a problem if the airway is narrowed too much (e.g. with poor abduction of the opposite vocal fold). Research cites a fairly variable rate and extent of fat resorption, so long-term results are unpredictable. According to some data, clinical improvement in voice is present for 26 months after the procedure. The survival of autologous fat depends to a large extent on the technique of obtaining it and injecting it into the vocal fold (12). Interesting is a study by Cantarella and colleagues in which fat was obtained by liposuction with a thin needle under low negative pressure, centrifuged and injected into the vocal fold in several layers. The voice quality improved in all subjects after injection, and results remained stable for 3–10 years after surgery (19).

The fat for injection can be obtained by liposuction or by cutting out a part of the subcutaneous fat, which is cut into smaller pieces, 2-3 $mm^2$ in size, with a scalpel or scissors. Most often we opt for the area around the navel, while alternative donor sites are the inner side of the thigh, the back side of the proximal part of the upper arm, or the neck, buccal or infrapatellar fat pads. In addition to vital fat cells, the fat obtained from liposuction also contains blood, damaged adipocytes and free fatty acids. The latter cause oxidative stress and therefore shorter survival of autologous fat. So, we have to remove them by rinsing with saline or centrifugation. The latter is also thought to increase the concentration of stem cells and angiogenic growth factors in fat and prolong its existence. Some authors recommend placing pieces of fat in a container with a suspension of human insulin for about 5 minutes, which stabilizes the adipocyte membrane. The fat prepared in this way is then injected into the vocal fold. The procedure is performed under general anesthesia, and the paraglottic area is viewed with a microscope after insertion of the laryngoscope. Due to the resorption and drainage of fat from the vocal fold after the procedure, we always make a 30% hypercorrection. After the procedure the patient is prescribed voice rest (20,21,22).

**The fascia lata** is the connective sheath that encircles the thigh muscles. Because it is the body's own tissue, there is no risk of disease transmission or immune response. After cutting it out from the anterolateral part of the thigh, we chop it into small pieces with a scalpel or scissors to get the consistency of a paste and inject it into the vocal fold. Autologous fascia gives good long-lasting results and remains stable in

the vocal fold 3–10 years after the procedure. Histological studies in dogs have shown that the fascia remains a stable collagen structure with abundant blood flow for many years. The implantation of the fascia into the Reinke's space at the scar and sulcus on the vocal fold also gives promising results (23).

**Calcium hydroxyapatite** (Radiesse®) is a mineral found naturally in bones and teeth, so it has great biocompatibility. Nevertheless, the literature describes a case where a foreign body granuloma was formed after injecting this substance. It is a ready-made synthetic material that can be safely injected on an outpatient basis under local anesthesia. It successfully corrects incomplete glottis closure and permanently improves voice quality. Due to the high viscosity, superficial injection is avoided. Studies in animal models have shown that even after 12 months it is practically not resorbed. However, its carrier (carboxymethylcellulose) is resorbed, so hypercorrection is required (14,15). A study by Thomas and colleagues confirms the long-term effect of calcium hydroxyapatite, with satisfactory results in patients 8–36 months after injection and a mean duration of 18.6 months (24).

**Teflon** does not degrade in the vocal fold, so it has been used for many years to reduce glottis insufficiency. However, long-term analyses have shown that foreign body granulomas may form several years after injection. In addition, it also passes into the surrounding tissues, so it is rarely used today (15).

## 7 Tissue engineering

A great potential in the treatment of vocal fold scarring in particular is represented by the growing advances in tissue engineering. The goal of tissue engineering is to obtain a substance that could restore the composition of the natural extracellular matrix of the lamina propria and restore the biomechanical properties of the vocal fold altered by scarring after injury, as well as prevent or limit fibrosis in the acute phase of vocal fold injury. Hyaluronic acid is one of the most studied substances in tissue engineering due to its role in healing. Studies in animal models have shown that by injecting various growth factors (hepatocyte growth factor, epidermal growth factor, basic fibroblast growth factor, transforming growth factor beta 1), stem cells and autologous fibroblasts into the lamina propria of the vocal fold immediately after the injury we reduce collagen deposition and the breakdown of hyaluronic acid, thus preventing or limiting the formation of scars. The same effect was achieved in the treatment of already formed scars, as compared to the untreated control group, better viscoelastic properties of the vocal fold, greater amplitude of vocal fold oscillations, better propagation of the mucosal wave through the vocal fold and reduction of glottis insufficiency were observed. Despite the encouraging results, additional controlled human studies are also needed for the safe use of tissue engineering methods in everyday clinical practice (4,25).

## 8 Treatment of glottis insufficiency at the University Department of Otorhinolaryngology and Cervicofacial Surgery

At the University Department of Otorhinolaryngology and Cervicofacial Surgery in Ljubljana, since 2006 we have been using the injection of autologous fat into one or both vocal folds to reduce glottis insufficiency. Below is a retrospective review of treatment success in those patients in whom we also performed acoustic analysis of voice samples to assess voice quality.

### 8.1 Methods

The study on the treatment of glottis insufficiency included patients who were

treated for glottis insufficiency at our clinic from 2010 to and including 2018, by injecting their autologous fat into one or both vocal folds. For the subjective and objective assessment of treatment success, we specifically analysed only those patients in whom, in addition to the subjective assessment of voice, swallowing and breathing problems before the procedure and 6–12 months after the procedure, an acoustic analysis of voice samples was performed.

From the medical documentation of patients, we summarized their gender, age at the onset of glottis insufficiency, its cause, subjectively expressed problems with voice, swallowing and breathing, stroboscopic examination and measured parameters of acoustic analysis of voice. Prior to the procedure, patients rated their voice, swallowing, and breathing problems as present or absent, and after the procedure as improved, unchanged, or worsening. Before and 6–12 months after the procedure, patients underwent acoustic analysis of voice samples of the vowel /a/ at the most comfortable pitch and volume. We used the Multi-Dimensional Voice Program (KayPENTAX, Montvale, New Jersey, USA). The analysis took into account the fundamental laryngeal frequency ($F_0$), pitch perturbation (jitter), amplitude perturbation (shimmer) and soft phonation index (SPI), which objectively indirectly assesses the contact between vocal folds.

In patients, subcutaneous fat was removed from the umbilical region under local anesthesia. It was then prepared for injection and injected into one or both vocal folds under general anesthesia by direct laryngoscopy under microscopic control.

Data were statistically processed using the SPSS program, version 22 (IBM Corporation, Armonk, New York, USA). We used the Shapiro-Wilk test of normal distribution, the paired t-test, the Wilcoxon test, the chi-square test, the independent samples t-test, and the Mann-Whitney test. For the limit of statistically significant differences, we determined $p < 0.05$.

## 8.2 Results

From 2010 to and including 2018, we injected autologous fat in 68 patients, of which 39 were men and 29 were women. At the onset of glottis insufficiency, they ranged in age from 14 to 84 years, averaging 55.74 years, and the standard deviation was 14.23 years. Glottis insufficiency was caused by vocal fold paresis or paralysis in 37 patients, sulcus vocalis in 22 patients, in 6 patients it occurred after irradiation due to laryngeal cancer, in 2 patients the cause was cricoarytenoid joint injury, and in one patient it was chordectomy due to vocal fold cancer. In six of them, the procedure had to be repeated 1-6 years after the procedure due to deterioration of the voice.

Acoustic analysis of the voice before and after the procedure was performed in 25 patients, of whom 12 were men and 13 were women. At the time of the damage, they ranged in age from 37 to 75 years, averaging 53.36 years with a standard deviation of 12.27 years. The cause of incomplete contact between the vocal folds was sulcus on one or both vocal folds in 8 patients, and in others the mobility of the vocal folds was disturbed. This was due to damage to the cricoarytenoid joint in one patient and neurogenic paresis or paralysis of the vocal fold in 16 patients.

All 25 patients had subjective voice problems prior to the procedure, and 6 and 12 months after the procedure, all of them described improved voice. Prior to injection, 6 patients had problems with aspiration during swallowing, and in all of them glottis insufficiency was due to paresis or paralysis of the vocal folds. After the procedure, only one other patient had such problems. No patients described breathing problems before or after the procedure.

The stroboscopic examination before the procedure showed the absence of any contact between the vocal folds in 9 patients, in the remaining 16 the contact was

present but incomplete. 6 or 12 months after injecting fat into the vocal fold, there was no contact between the vocal folds in 4 patients, in 4 patients the contact was complete but short, and in others the contact was incomplete, but the gap between the vocal folds was significantly smaller than before the injection.

Acoustic analysis of voice patterns showed an improvement in all parameters after injecting fat into the vocal fold. A statistically significant difference was observed only in the measurement of pitch pertubation (jitter). The results are presented in Table 1.

Sulcus on the vocal fold was present in 22 patients with glottis insufficiency, of whom 12 were men and 10 were women. The analysis did not show a statistically significant increase in frequency of the occurrence of sulcus on the vocal fold in either sex ($p = 0.746$).

There was no statistically significant difference between the parameters of acoustic analysis of voice depending on the cause of glottis insufficiency (paresis, sulcus) ($F_0$ - $p = 0.499$; jitter - $p = 0.582$; shimmer - $p = 0.270$; SPI - $p = 0.378$).

Four patients attended voice therapy before and after the vocal fold augmentation with autologous fat, 5 patients only after the procedure, and the remaining 16 did not receive speech therapy.

## 9 Discussion

Both the subjective assessment of the patients and the acoustic analysis of the voice confirmed the success of the injection of autologous fat in the treatment of incomplete closure of the vocal folds.

Team treatment of patients with glottis insufficiency includes the work of a clinical speech therapist. We consider injection if the speech therapy is not successful or if the gap between the vocal folds in phonation is so large that we justifiably do not expect improvement only with voice therapy. Speech therapy is also necessary as soon as possible after injecting autologous fat into the vocal fold, so that the patient acquires the correct phonation technique in the new anatomical conditions. The results of glottis insufficiency treatment were analysed during the period of staff shortages at the Centre for Voice, Speech and Swallowing Disorders, when unfortunately, a speech therapist was not available to treat a certain number of patients. We anticipate that the inclusion of a clinical speech therapist before and after injecting autologous fat into one or both vocal folds would further increase or improve the subjective speech perception.

## 10 Conclusion

Medialization of the vocal fold by injection is an effective method of reducing mild to moderate glottis insufficiency for a variety of reasons. There are many possible approaches and materials for injection. Each of them has its advantages and disadvantages, which must be carefully con-

**Table 1:** Results of acoustic voice analysis in 25 patients with glottic insufficiency before and after autologous fat injection into vocal folds.

| Parameter | Before injection | After injection | p |
|---|---|---|---|
| $F_0$ [Hz] | 210.42 | 189.66 | 0.081 |
| pitch perturbation (jitter) [%] | 4.87 | 2.38 | 0.009 |
| amplitude perturbation (shimmer) [%] | 7.98 | 5.95 | 0.109 |
| SPI | 23.51 | 23.11 | 0.798 |

$F_0$ – fundamental frequency, SPI – soft phonation index

sidered and taken into account for optimal treatment success. The method of treatment should be tailored to each patient. In doing so, it is necessary to take into account the cause of his or her voice problems, their severity and the patient's personal expectations. It is also necessary to explain to the patient that it is difficult to expect the quality of the voice to return to the level before the damage, but the voice can be significantly improved. In recent years, a lot of research has been done in the field of strengthening the vocal fold by injection, which promises to be even more successful in the future. Especially encouraging are the rapidly evolving tissue engineering methods for the treatment of vocal fold scarring.

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