Regenerative medicine approaches for the management of respiratory tract fistulas

Angelo Trivisonno1†, Dania Nachira2†, Ivo Boškoski3†, Venanzio Porziella2†, Giuliana Di Rocco4, Silvia Baldari4 and Gabriele Toietta4*†

Abstract
Respiratory tract fistulas (or fistulae) are abnormal communications between the respiratory system and the digestive tract or the adjacent organs. The origin can be congenital or, more frequently, iatrogenic and the clinical presentation is heterogeneous. Respiratory tract fistulas can lead to severely reduced health-related quality of life and short survival. Therapy mainly relies on endoscopic surgical interventions but patients often require prolonged hospitalization and may develop complications. Therefore, more conservative regenerative medicine approaches, mainly based on lipotransfer, have also been investigated. Adipose tissue can be delivered either as unprocessed tissue, or after enzymatic treatment to derive the cellular stromal vascular fraction. In the current narrative review, we provide an overview of the main tissue/cell-based clinical studies for the management of various types of respiratory tract fistulas or injuries. Clinical experience is limited, as most of the studies were performed on a small number of patients. Albeit a conclusive proof of efficacy cannot be drawn, the reviewed studies suggest that grafting of adipose tissue-derived material may represent a minimally invasive and conservative treatment option, alternative to more aggressive surgical procedures. Knowledge on safety and tolerability acquired in prior studies can lead to the design of future, larger trials that may exploit innovative procedures for tissue processing to further improve the clinical outcome.

Keywords: Adipose tissue, Fistula, Regenerative medicine, Respiratory tract, Lipotransfer, Mesenchymal stromal cells, Head and neck, Tracheoesophageal fistula, Minimally invasive treatments, Airway defects restoration

Introduction
Fat grafting, referred also as lipotransfer, involves harvesting of adipose tissue, processing of the collected fat to eliminate oil, liposuction fluids, and blood components, and then re-injection of the manipulated tissue into the area that needs treatment [1]. The first documented surgical fat grafting procedure dates back to 1893 when Gustav Neuber described the transfer of adipose tissue harvested from the forearm into the periorbital region to correct a depressed scar [2]. In 1987, Sydney R. Coleman developed an innovative technique of liposuction allowing for adipose tissue harvest under local anesthesia with less extensive damage [3]. The procedure of fat grafting has been broadly explored to repair soft tissue volume loss (reconstructive surgery) and to enhance cosmetic appearance (cosmetic surgery) [3, 4]. More recently, fat grafting has also been used to promote tissue or organ healing (regenerative medicine) [1, 5–7]. Several parameters such as fat preparation, implantation techniques, and recipient site may affect graft retention [8]. As a result, in the absence of a general consensus on a standardized procedure, the clinical outcome of lipotransfer is not always predictable [9, 10].

* Correspondence: gabriele.toietta@ifo.gov.it
† Angelo Trivisonno, Dania Nachira, Ivo Boškoski and Venanzio Porziella contributed equally to this work.
4 Department of Research, Advanced Diagnostic, and Technological Innovation, Translational Research Area, IRCCS Regina Elena National Cancer Institute, via E. Chianesi 53, 00144 Rome, Italy
Full list of author information is available at the end of the article

© The Author(s). 2020 Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
In 2001, Zuk et al. demonstrated the presence within the adipose tissue of multipotent cells able to differentiate in vitro into adipogenic, chondrogenic, myogenic, and osteogenic cells [11]. This discovery provided further support for the perspective use of adipose tissue-derived material for regenerative purposes [12, 13]. The isolation of multipotent cells from the adipose tissue complex involves several steps: (1) fat digestion by a solution containing collagenase, (2) elimination of tissue debris by filtration, (3) centrifugation to collect the cellular component of the so-called stromal vascular fraction (SVF), (4) expansion of the isolated cells in culture to obtain adipose tissue-derived mesenchymal cells, and (5) flow cytometry analysis for phenotypic characterization of the isolated cells [14]. According to the definition released by the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT), uncultured SVF cells are a heterogeneous population that includes stromal cells, endothelial cells, erythrocytes, fibroblasts, lymphocytes, monocyte/macrophages, and pericytes [15, 16]. Mesenchymal stromal/stem cells (MSC), referred also as adipose tissue-derived stromal cells (ASC), are characterized by rapid plastic adherence in culture; moreover, they express the phenotypic markers CD90, CD73, CD105, and CD44, while they are negative for CD45 and CD31 expression; in addition, MSC can differentiate into osteocytes, adipocytes, and chondrocytes in vitro in the presence of appropriate inductive media [15]. During the course of the years, adipose tissue-derived multipotent cells [11] were named also as stem cells [17], as mesenchymal stromal cells [15] and, more recently, as medicinal signaling cells [18], maintaining the MSC acronym [16]. The evolution of the nomenclature reflects a paradigm shift on how MSC are believed to exert their therapeutic effect in regenerative medicine procedures. In facts, the term “multipotent stem cells” was originally coined to imply that MSC might differentiate into cells which directly participate into tissue healing (building block activity). Several experimental and clinical evidences subsequently indicated that, despite the development of different strategies aiming at improving cell engraftment [19], the number of cells which are actually able to survive and persist upon transplant, to differentiate in vivo and to take part in tissue regeneration are far too low to justify the clinical benefit observed in cell therapy procedures [20]. Therefore, the attention was pointed to the ability of MSC, as “medicinally signaling cells”, to produce trophic, immunomodulatory factors, either directly or via extracellular vesicles, which might promote tissue regeneration and/or tissue stem cells homing (paracrine activity) [21]. The exact molecular mechanism(s) underlying the regenerative potential associated with adipose tissue- and cell-based therapies still require complete elucidation [22]. MSC are believed to exert their pro-healing function mainly through the release of paracrine factors and extracellular vesicles that may stimulate the migration and activation of local tissue-specific stem cells that contribute to tissue regeneration, promotion of neo-angiogenesis, modulation of inflammatory and immunomodulatory responses, and increase of anti-oxidative and anti-apoptotic effects [23, 24]. Several regenerative medicine clinical trials using MSC cell transplant procedures have been performed [25]; in particular, treatment of perianal fistulising Crohn’s disease, a chronic inflammatory disorder of the gastrointestinal tract, using cell therapy has been extensively investigated in virtue of the immunomodulatory properties of MSC [26–28]. A phase III study verified the safety and the efficacy in long-term closure of perianal fistulas by local injection of adipose tissue-derived MSC [29]. Placement of esophageal stent [30] or bioprosthesis materials [31] are currently used for the management of different esophago-respiratory fistulas. Unfortunately, this kind of surgical intervention often requires long hospitalization and may be associated with a considerable risk of adverse events. Recently, the therapeutic efficacy of the delivery of cell and tissue-based products for the treatment of fistulas of different etiology has been studied. We performed a narrative literature review on the management of different kinds of fistulas and esophageal and airway defects through the administration of cellular and tissue-based products, as a conservative alternative procedure to more aggressive surgery. We then focus on the possible future directions, including the potential use of different methods of adipose tissue manipulation, which may provide an opportunity to improve the clinical outcome of the procedure. In order to identify the studies evaluating the effects of autologous fat grafting and/or mesenchymal stromal cell therapy on airway tissue defects, we interrogated PubMed, Web of Science, Scopus, and Google Scholar electronic databases. Moreover, we consulted the ClinicalTrials.gov trial registry. We conducted literature search by combining Medical Subject Headings terms such as “respiratory tract fistula”, “mesenchymal stromal cell”, “adipose tissue-derived stromal cells”, “stromal vascular fraction”, “lipoaspirate”, and “adipose tissue”. Studies were not constrained by publication date or publication status. Only clinical studies written in English were examined. Identified articles were mainly case reports since no clinical study with a large sample size has been evaluated so far (Table 1). Moreover, the nature of the disease, as well as the method of processing and local administration of the material, predominantly derived from autologous adipose tissue, varied among the studies.
Clinical application of adipose tissue-derived material for the treatment of respiratory tract fistulas

According to the Medical Subject Headings (MeSH) definition, a respiratory tract fistula is “an abnormal passage communicating between any component of the respiratory tract or between any part of the respiratory system and surrounding organs”. If left untreated, respiratory tract fistulas are associated with high mortality rates [44–46]. The most common interventional therapy relies on stent placement. In addition, more conservative strategies based on regenerative medicine approaches have also been considered. In the following sections, we briefly review some of the tissue/cell-based clinical studies described for the management of various types of respiratory tract fistulas (Table 1).

Oroantral fistula
An oroantral fistula (OAF) is a pathologic communication between the oral and the antral cavities. The removal of the maxillary posterior teeth is considered the major cause of OAF development. Small-size OAF tend to heal spontaneously, while surgical intervention is recommended for fistulas larger than 3 mm. Taking into consideration the size of the OAF and the condition of the surrounding tissues, different therapeutic approaches have been evaluated [47]. Larger defects, such as the ones subsequent to tumor resection, may require the use of autogenous bone and soft tissue grafts, the placement of allogenic materials or xenografts. Flaps utilizing local tissue, such as buccal and palatal flaps, can be used to close moderate-sized defects. In particular, application of the buccal fat, a lobulated form of adipose tissue, has been quite extensively utilized since its description in 1977 [32–34]. The adipose tissue used to repair OAF is generally coated by the surrounding mucosa in 4 to 6 weeks, thus promoting complete epithelialization of the treated area [48].

Pharyngocutaneous fistula
Pharyngocutaneous fistula (PCF) is a pathological communication involving the digestive tract and the skin of the neck. PCF is a quite common complication after head and neck surgery [49]. Presence of PCF may prolong recovery and delay adjuvant oncologic treatments. The majority of cases are treated with conservative management in order to promote spontaneous healing, but approximately 30% of patients require a more aggressive surgical intervention. Two different case reports have described successful PCF healing by fat grafting in patients undergone to partial pharyngectomy [35, 36]. In particular, in the case report described by Hespe et al., two rounds of autologous fat grafting delivered into the area immediately surrounding the PCF using both blunt cannulas and 18 gauge needles were performed to achieve complete fistula healing [36]. Conversely, Sapundzhiev et al. reported a case report of a patient administered with autologous fat around the internal opening of the PCF with a Peretti angular injection cannula using an endoscopic access to the neopharynx [35].

Tracheoesophageal fistula
Tracheoesophageal fistulas (TEF) are connections between the airway and upper gastrointestinal tract; they need prompt identification and treatment to prevent recurrent and intractable infections due to tracheobronchial contamination. TEF are broadly categorized into congenital and acquired fistulas, the latter group being further divided into nonmalignant and malignant. Congenital TEF occur in 1 in 3,000–5,000 live births [50], and they are usually diagnosed within the first year of life, while presentation in adults is rare [51]. Acquired nonmalignant TEF are mainly associated with traumatic injury, foreign body or caustic ingestion [52]. The majority of acquired nonmalignant TEF are mostly due to compression from an inflated endotracheal or tracheostomy tube cuff which may occur in approximately 0.5% of patients undergoing tracheostomy or intermittent positive

Table 1 Reports of therapeutic procedures involving fat or mesenchymal stromal cells for the management of respiratory tract fistulas

| Condition                  | Intervention                                                                 | Patients enrolled | Reference |
|----------------------------|------------------------------------------------------------------------------|-------------------|-----------|
| Oroantral fistula          | Autologous buccal fat pad                                                    | 1+ 25             | [32–34]   |
| Pharyngocutaneous fistula  | Autologous fat                                                               | 1 + 1             | [35, 36]  |
| Tracheoesophageal fistula   | Autologous fat                                                               | 1                 | [37]      |
| Tracheomediastinal fistula  | Autologous adipose tissue SVF in fibrin glue                                 | 1                 | [38]      |
| Bronchopleural fistula     | Autologous adipose tissue-derived MSC-seeded matrix graft                    | 1                 | [39]      |
|                           | Autologous bone marrow-derived MSC                                           | 1 + 2             | [40, 41]  |
|                           | Umbilical cord MSC                                                           | 1                 | [42]      |
|                           | Autologous fat                                                               | 8                 | [43]      |

SVF stromal vascular cells (uncultured), MSC mesenchymal stromal cells
pressure ventilation [53] (Fig. 1). Occasionally, non-malignant acquired TEF may arise from local inflammation and infection, such as tuberculosis and granulomatous infection [54].

Malignant acquired TEF have been associated with several types of cancers. In particular, TEF incidence has been reported as 4.5% following primary malignant esophageal tumors and 0.3% in primary malignant lung tumors [55]. Tumor invasion and cancer-related tissue necrosis may contribute to the pathogenesis of malignant TEF. In addition, also chemoradiotherapy and anti-angiogenic therapy, affecting local architectural and vascular tissue changes, can increase the risk of TEF formation [46] (Fig. 2). In this regard, cell-based therapies may attenuate chemotherapy-induced tissue injuries [56].

Small size TEF may close spontaneously, while fistulas over 20 mm in size are associated with poor survival [57]. Therefore, prompt therapeutic intervention is needed in order to arrest the contamination of the airway and enabling normal oral alimentation. Different approaches have been developed for the management of both acquired non-malignant [57] and malignant TEF [44, 46, 55]. Surgical interventions include esophageal stent placement, bypass, resection, and surgical repair. Conservative treatments, alternative to surgical procedures, mainly consist of supportive care to prevent contamination of the respiratory tract. Moreover, use of
autologous tissue-assisted regenerative procedure may represent a valuable therapeutic option. In this regard, it has been described a case report of a 55-year-old man affected by congenital TEF successfully treated with local injection of autologous fat using a pressurized injection device [37]. Long-term complete healing was observed after two sessions of administration of autologous fat and the patient remained asymptomatic more than 10 years.

Tracheomediastinal fistula
A tracheomediastinal fistula (TMF) is a communication between the trachea and the mediastinum. TMF formation is rare and generally associated with airway tumors. Díaz-Agero Álvarez described a case report of TMF, subsequent to endoscopic laser therapy of tracheal cancer, treated with bronchoscopic administration of autologous ASC in fibrin glue suspension [38]. In particular, autologous ASC were isolated by collagenase digestion from 150 ml of lipoaspirate. Then, approximately 5.0 × 10^6 cells were mixed in fibrin glue and injected through a bronchofibroscope into the cavity of a 2-cm^2 TMF. One-year follow-up showed complete closure of the fistula with re-epithelialization and neovascularization of the area (Fig. 3) [38].

Bronchopleural fistula
A bronchopleural fistula (BPF) is defined as a pathological communication between the bronchial tree and the pleural space [58]. BPF is a severe postoperative complication of pneumonectomy or other pulmonary resection interventions with high rates of morbidity and mortality (Fig. 4). Therefore, surgical or bronchoscopic interventions are needed to promote BPF closure.

As conservative alternative to more traumatic surgical procedures, administration of mesenchymal cells has been performed in order to promote healing of the tissue surrounding the fistula [42, 59]. In particular, Petrella et al. described an approach of autologous bronchoscopic perilesional transplantation of ten million bone marrow-derived mesenchymal cells for the treatment of a small-caliber (3 mm) BPF developed in a 42-year-old man after right extra pleural pneumonectomy for malignant mesothelioma [40]. Aho et al. described a case report of a 66-year-old patient with a large (1.5 cm) BPF treated with a matrix graft seeded with autologous mesenchymal stem cells. Cells were obtained by collagenase digestion from autologous adipose tissue and underwent three passages of amplification in vitro, and finally, 2.5 × 10^7 MSC were seeded on a matrix of synthetic bio-absorbable poly(glycolide:trimethylene carbonate) copolymer under Good Manufacturing Practices (GMP) procedures. Five days after cell seeding, the matrix graft was surgically placed over the BPF to promote healing [39]. The treated patient remained asymptomatic during the clinical follow-up of 1.5 years. Díaz-Agero Álvarez et al. described the treatment of two patients suffering from BPF by bronchoscopic administration of adipose tissue-derived stromal cells (ASC) isolated by collagenase digestion and not expanded in culture [41]. One patient, affected with a 6-mm diameter BPF, was administered with 4.0 × 10^6 ASC leading to 80% closure of the fistula. Six months later, the procedure was repeated with the administration of additional 5.0 × 10^6 ASC to achieve full healing. The second patient, who had a 3-mm diameter fistula, received 1.3 × 10^7 ASC in a single procedure. Patients were observed for a 3-year follow-up, and no treatment-related adverse effects were reported [41]. Recently, Zeng et al. described a case report of successful closure of a BPF (5 × 2 mm) resulting from lobectomy, treated by administration through a flexible bronchoscope of 2.0 × 10^7 umbilical cord MSC around the fistula [42]. A computed tomography scan performed 6 months after the treatment revealed fistula healing and the BPF did not relapse during the 2-year follow-up. A different approach has been evaluated by Hurramoto et al. in lung cancer patients undergoing lobectomy. The authors suggest that the use of isolated pericardial fat tissue to close the bronchial stump might prevent the occurrence of BPF [60]. Recently, endoscopic administration of autologous fat was performed for the treatment of BPF in 8 patients and resolution was observed in all cases [43].

Clinical application of adipose tissue-derived material to promote tissue regeneration in the oropharyngeal tract
In the next sections, we present a brief overview of the studies of reconstructive/regenerative surgery assessing local administration of fat or adipose tissue-derived mesenchymal stromal cells to restore tissue loss or damage in the oropharynx (Table 2).

Tracheoesophageal puncture
Tracheoesophageal puncture (TEP) with voice prosthesis placement is an extensively used technique to restore vocal function in patients undergoing total laryngectomy and pharyngolaryngectomy. One of the most frequent complications of this procedure, which usually requires to replace the voice prosthesis, is enlargements of the puncture, with leakage of saliva or food [76]. Administration of autologous fat around the puncture has been described as an effective and safe procedure, which allows the conservation of the voice prosthesis, by promoting the increase of the thickness of the tracheoesophageal wall [61]. In particular, 4 out of the 10 treated patients maintained long-term (up to 65 months) tracheoesophageal speech with no leakage.
Hypertrophic tracheostomy scar

Hypertrophic scar formation at the site of tracheostomy is quite frequent. The scar tissue may attach to the trachea causing discomfort during the act of swallowing. Several surgical options have been described for hypertrophic scar ablation [77]. Fat, adipose tissue-derived mesenchymal stromal cells, and stromal cell-derived factors possess antifibrotic functions which exert a positive role in difficult scar treatment [78]. A minimally invasive procedure for the treatment of post-tracheostomy hypertrophic scar by means of intraleisional administration of adipose tissue has been employed by Mazzola et al. resulting in valuable cosmetic results and in improved skin quality and texture [62]. All 10 treated patients enrolled in the study achieved satisfying esthetic and functional improvements.

Head and neck reconstruction after radiotherapy

Reconstructive surgery may be required for functional and cosmetic soft tissue restoration in patients with head and neck cancers. Actually, treatment of head and neck cancers may require the surgical removal of a large

---

Fig. 3 Bronchoscopic and CT images from the region of the fistula. 

(a) Bronchoscopic image recorded before cell therapy. The fistula can be seen on the anterior tracheal wall which had been totally destroyed after the laser treatment of the tumor. The entrance was about 10 mm in diameter and the bronchoscope could pass through it. Inset: Anthracotic mediastinal lymph nodes as seen through the wall of the fistula. 

(b) CT image recorded before cell therapy. The fistula was situated between the trachea and a pretracheal mediastinal cavity with an area of 2 cm², next to the superior vena cava and pulmonary artery, near the ascending aorta. 

(c) Bronchoscopic image recorded 1 year after cell therapy. The entrance to the fistula was much smaller (diameter 3–4 mm). Inset: The walls of the fistula were covered with “new” epithelium and vessels as a result of neovascularisation and epithelialisation. 

(d) CT image from the same region of the fistula 1 year after cell therapy. One year after treatment the cavity had disappeared. 

(e) CT image from the region of the fistula recorded 1 year after cell therapy. This image is the only one to show remnants of the previous fistulous tract. It is clear that the fistula had closed. *, small depression; VC, superior vena cava; AOa, ascending aorta; AOd, descending aorta; PA, right pulmonary artery; LPA, left pulmonary artery. Reproduced with permission from Díaz-Agero Alvarez et al. [38]
amount of tissue surrounding the tumor; moreover, adjuvant therapeutic irradiation may result in extensive tissue damage and induction of radiation-induced skin fibrosis. Administration of autologous fat has been evaluated as a suitable method to achieve both esthetic and functional reconstruction in head and neck oncologic patients, partially restoring volume loss, reducing excessive scar formation and radiation-induced skin fibrosis in the treated areas [63–65, 79]. More than 60 patients have been treated using autologous fat administration in three different studies [63–65, 79]. Some concerns have been raised about the possibility of administered fat to promote residual tumor cell invasion and metastasis [80]. Preliminary data obtained using head and neck cancer cell lines both in vitro and in vivo suggest that the procedure may be safe, but further investigation performed on patient-derived tumor samples is needed [81].

Velopharyngeal insufficiency

Velopharyngeal insufficiency (VPI) occurs when there is incomplete velopharyngeal closure. As reviewed by Nigh et al. administration of autologous fat has been collectively performed for the management of VPI in more than 250 patients [66]. Recently, the procedure was described for additional 11 adult patients [67]. Based on these preliminary studies, the procedure of injection pharyngoplasty with autologous fat could be considered as a safe and effective treatment option for mild VPI.

Vocal fold scars

Vocal fold scars are scarring and fibrotic formations on the layer of the vocal cord. Preclinical studies support the rationale for using cell therapy for the treatment of vocal fold scarring which may occur as a result of surgical or iatrogenic injury [82]. A clinical team at the Hopitaux De Marseille, France, promoted a clinical trial entitled “Innovative treatment for scarred vocal cords by local injection of autologous stromal vascular fraction” (NCT02622464), described the first clinical case report [68], and have recently published the results on additional 8 patients [69]. The therapeutic intervention consisted in autologous adipose tissue harvest, enzymatic digestion, isolation of ASC under GMP conditions, and same-day local administration at the laryngeal level of

Table 2 Reports of therapeutic procedures involving fat or adipose tissue-derived cells to promote tissue regeneration in the oropharyngeal tract

| Condition                                      | Intervention       | Patients enrolled | Reference   |
|------------------------------------------------|--------------------|------------------|-------------|
| Tracheoesophageal puncture                      | Autologous fat     | 10               | [61]        |
| Hypertrophic tracheostomy scar                 | Autologous fat     | 10               | [62]        |
| Radiation-induced fibrosis and volume defects in head and neck oncology | Autologous fat | 38 + 11 + 12 | [63–65]        |
| Velopharyngeal insufficiency                   | Autologous fat     | 11 + 251         | [66, 67]    |
| Vocal fold scars                               | Autologous SVF     | 8 + 1            | [68, 69]    |
|                                               | Autologous fat     | 24               | [70]        |
|                                               | Autologous nanofat and microfat | 7 | [71] |
| Unilateral laryngeal nerve paralysis           | Autologous fat     | > 90             | [70, 72–75]|

SVF adipose tissue-derived stromal vascular fraction cells (uncultured)
Follow-up analysis performed at 12 months indicated an improvement in the voice handicap index score without serious adverse events causally related to the treatment. In addition, Cantarella et al. described the treatment of 7 patients with vocal fold scarring [70]. More recently, the same authors described the treatment of 7 patients with vocal fold scarring by nanofat and microfat grafting [71]. In particular, in this group of patients, microfat was administered deeply in the vocal fold and nanofat emulsion was injected in the most superficial layer of the vocal fold in the scarred tissue. Follow-up analysis performed at 3 months indicated improvements in the voice handicap index.

**Unilateral recurrent laryngeal nerve paralysis**

Unilateral recurrent laryngeal nerve paralysis may occur secondary to injury of the recurrent laryngeal nerve due to cancers, trauma, and surgery. In 1991, Mikaelian et al. published a preliminary report describing a procedure of autologous fat injection into a paralyzed vocal cord in 3 patients affected by unilateral vocal cord paralysis [72]. Since then the procedure has been performed on several additional cases describing long-term (>1 year) improvement of vocal parameters after a single fat injection [70, 73–75, 83, 84]. In particular, in a clinical trial (NCT02904824), a group of patients was treated by administration of adipose tissue and a second group with the same amount of adipose tissue in presence of a not-well quantified amount of ASC (cell-assisted lipotransfer) [74]. However, the study was inconclusive in determining any definite difference between the clinical outcomes of the two groups [74].

**Future directions**

We have summarized in Tables 1 and 2 a number of published clinical studies on promotion of tissue healing in the respiratory tract applying a conservative, regenerative medicine-based approach. Most of the evidence-based data have been collected from single case reports or series of case studies. As a matter of facts, the design of larger, multisite clinical trials is hampered by the relatively small number of affected individuals that can be enrolled and by the lack of clinical institutions which have sufficient knowledge and resources for innovation implementation in this field. Accordingly, the number of clinical trials which have been performed or are currently ongoing is still limited (Table 3). Bone marrow, adipose tissue, and umbilical cord blood are the most frequently utilized sources of MSC for clinical trials [23], including the ones described for fistula healing and tissue regeneration in the oropharynx (Table 3). Bone marrow and adipose tissue are replenishable sources of MSC suitable for autologous transplant [85]. Bone marrow collection is an invasive procedure, while subcutaneous adipose tissue can be easily harvested [86]. Adipose tissue contains up to 500 more MSC cells than an equivalent amount of bone marrow; moreover, adipose tissue-derived MSC can be easily expanded in vitro since they have higher proliferation rate compared to bone marrow-derived MSC [87]. In addition, MSC derived from adipose tissue promote stronger immunosuppressive effects than MSC isolated from other sources [88]. MSC in umbilical cord are rare but can be amplified in vitro given that can undergo to more cell divisions than MSC from adult tissues before reaching senescence. Optimal storage of the cryopreserved umbilical cord tissues or MSC is required for autologous use. In general, easiness of collection and processing make adipose tissue

| Condition | Intervention | ClinicalTrials.gov Identifier |
|-----------|-------------|-------------------------------|
| Tracheoesophageal fistula, bronchoesophageal fistula, tracheal fistula | Adipose-derived stromal vascular fraction for aero-digestive fistulae | NCT03792360 |
| Bronchial fistula | Human amniotic epithelial cells for treatment of bronchial fistula | NCT02959333 |
| Bronchopleural fistula | Umbilical cord mesenchymal stem cells for treatment of bronchopleural fistula | NCT02961725 |
| Enterocutaneous fistula | Stromal vascular fraction for treatment of enterocutaneous fistula | NCT01584713 |
| Dysphonia | Innovative treatment for scarred vocal cords by local injection of autologous stromal vascular fraction | NCT02622464 |
| Vocal cord paralysis, unilateral | Injection laryngoplasty using autologous fat enriched with adipose-derived regenerative stem cells | NCT02904824 |
| Hoarseness, dysphonia, aponia, vocal fold; scar | A study of local administration of autologous bone marrow mesenchymal stromal cells in dysphonic patients with vocal fold scarring | NCT04290182 |
| Vocal fold; scar | Pilot study of bone marrow stem cell treatment of patients with vocal fold scarring | NCT01981330 |

*Source: https://clinicaltrials.gov/, accessed July 2020*
the best source of material suitable for clinical studies aiming at the promotion of tissue healing in the respiratory tract. Collectively, the data acquired so far generally confirm the safety and suggest the occasional clinical efficacy of the delivery of adipose tissue-derived material for the treatment of respiratory-digestive tract fistulas [89]. Nonetheless, there is a strong need to optimize and standardize the protocols to process adipose tissue in order to improve the reproducibility of the procedure. Moreover, the follow-up conditions must be clearly defined to better evaluate the beneficial effects of the treatment.

The majority of the collected clinical information is based upon studies exploiting administration of unprocessed autologous adipose tissue collected by liposuction (Fig. 5; Tables 1 and 2) [90]. Adipose tissue is mainly composed of adipocytes, which constitute more than 90% of its volume; additional components of the stromal vascular fraction (SVF) include mesenchymal/stromal cells (MSC), preadipocytes, fibroblasts, endothelial cells, vascular smooth muscle cells, resident monocytes/macrophages, and lymphocytes. MSC have a perivascular origin; accordingly, MSC content is higher in vascularized hypodermic adipose tissue [86]. In consideration of the presence within the adipose tissue of cells able to differentiate and to promote tissue regeneration acting in a paracrine fashion [22, 91], fat has been recently reconsidered not only as a simple physical filler for cosmetic surgery procedures, but also as a source of "medically signaling cells" [18]. Therefore, as an alternative to or in conjunction with lipotransfer, transplantation of adipose tissue-derived cells has been clinically evaluated for the treatment of a variety of regenerative purposes [25]. In particular, in 2003, Garcia-Olmo firstly reported the effective treatment of rectovaginal fistula in Crohn’s disease by administration of autologous adipose tissue-derived MSC [92]. Subsequently, additional phase I to III clinical trials, collectively enrolling more than 300 Crohn’s patients, have been performed indicating that cell transplantation is safe and effective [26, 27, 29, 93]. Isolation of MSC cells mainly relies on collagenase digestion of the fat collected by liposuction [14]; from the regulatory point of view enzyme-based protocols cannot be considered “minimal manipulation” and therefore the manufacturing procedures are subjected to the regulation applied for the Advanced Therapies Medicinal Products (ATMPs) [94]. Moreover, amplification in culture of MSC requires GMP conditions, is time-consuming, and is associated with high cost and regulatory burden. Therefore, alternative, enzyme-free strategies to obtain a ready-to-use adipose tissue-derived material have been developed [95] and administration of mechanically isolated adipose tissue SVF has been performed for the treatment of different pathologies [96]. In particular, administration of homogenized adipose tissue has been recently proved effective in the treatment of perianal fistulas in patients with Crohn’s disease [97–99], providing a suitable alternative to MSC administration [100].

We believe that the strategy of micro-fragmented adipose tissue transplantation, considered for the promotion of healing of anal fistulas, may be effectively applied also for the management of fistulas affecting the upper esophageal tract (Fig. 5). Administration of micro-fragmented adipose tissue-derived stromal fraction tissue (tSVF) has numerous advantages compared to lipotransfer or administration of MSC: tSVF can be obtained from lipoaspirate by non-enzymatic methods with

![Fig. 5 Schematic representation (not in scale) of the therapeutic intervention procedure proposed for the management of respiratory tract fistulas by endoscopic delivery of autologous adipose tissue-derived material. Some templates to create this figure are used/adapted from Servier Medical Art (https://smart.servier.com/), available under a Creative Commons Attribution 3.0 Unported License](Image)
minimal manipulation; the procedure is rapid and cost-effective and can be performed intra-operatively [95]; in tSVF, the relative number of MSC per tissue volume is higher than in adipose tissue, since adipocytes, red blood cells, oil, and aqueous fractions have been discarded; homogenized tSVF can be precisely administered at the site of tissue damage through a 25-G needle; compared to enzymatically derived SVF, micronized tSVF retains the native extracellular matrix and perivascular structures, reducing induction of anoikis upon transplant [101]; microfat lobules are less prone to oxidative stress compared to unprocessed fat, thus improving graft retention since oxidative damage has a detrimental effect on survival of transplanted cell [102] and adipose tissue [103].

Several preclinical studies assessed the efficacy of MSC therapy for laryngotracheal stenosis [104]. Therapeutic benefit associated to MSC transplant is likely attributable to the secretion of soluble factors and to the release of extracellular vesicles (EVs) [22]. Indeed, administration of conditioned medium collected from MSC cell culture may be instrumental in stimulating resident bronchioalveolar stem cells, supporting tissue regeneration in the respiratory system [105]. Moreover, administration of extracellular vesicles produced by adipose tissue MSC mixed in a thermoresponsive gel has been shown to promote esophageal fistula healing in a porcine model [106]. Accordingly, the delivery of MSC secretome has been proposed as a therapeutic strategy for lung injury and acute and chronic diseases [107, 108]. Notably, micro-fragmented fat has improved paracrine anti-inflammatory, anti-fibrotic, and pro-angiogenic properties instrumental for supporting tissue regeneration compared to cultured MSC [109].

Combination of autologous mesenchymal stromal cells and tissue-engineered scaffolds is an interesting and rapidly evolving approach in the regenerative medicine arena, potentially suitable also to support the healing of large-size fistulas and partial or long-segment defects of the esophagus [110]. The ideal scaffold should be biocompatible and biodegradable, with a degradation rate similar to the tissue regeneration time. Placement of tissue-engineered graft has been mostly described in preclinical models: for instance, the use of suture filament embedded with adipose tissue-derived MSC has been used to promote fistula healing in a rat model [111]; promotion of esophageal anastomotic leakage healing has been achieved in rabbits by administration of fibrin scaffolds including MSC [112]; synthetic polyurethane electro-spun grafts seeded with autologous MSC have been tested for esophageal tissue remodeling in pigs [113]. To circumvent possible biocompatibility problems, esophagus-like scaffold-free structures embedded with MSC suitable for esophageal repair have been generated by 3D bioprinting and transplanted in rats [114].

In addition, accurate 3D-printed patient-personalized stent, based on 3D reconstruction of the fistula image, can be created, as assessed for the treatment of enterocutaneous fistulas [115]. Clinical translation of preclinical research on tissue engineering for airways defects has been so far limited but the rapid pace of the technological developments in tissue engineering and in 3D bioprinting can anticipate future therapeutic opportunities [116].

**Conclusions**

Respiratory tract fistulas may develop as complications in various surgical interventions, trauma, and accidental foreign body and caustic ingestion or, rarely, may be congenital. Small size (< 2 mm) fistulas generally heal spontaneously, while large caliber fistulas may be associated with severe, life-threatening complications. We reviewed several case reports suggesting that endoscopic local delivery of adipose tissue/MSC may represent a moderately invasive and a relatively safe treatment option, alternative to aggressive surgery, to promote fistula healing. One possible strategy which may provide a further therapeutic advancement could be represented by the delivery of micronized adipose tissue, which can be obtained with minimal manipulation [95]. However, much work remains to be performed before successfully translation of clinically competitive cell- and tissue-based new therapies for respiratory tract fistula healing. In particular, standardization of the procedures, optimization of clinical trial design, and guidance in follow-up analysis are needed in order to assess the long-term occlusion of the fistulas in the treated patients.

**Abbreviations**

ASC: Adipose tissue-derived stromal cells; ATMPs: Advanced therapies medicinal products; BPFF: Bronchopleural fistula; EV: Extracellular vesicles; GMP: Good Manufacturing Practices; MSC: Mesenchymal stromal cells; OAF: Oroantral fistula; PCF: Pharyngocutaneous fistula; SVF: Stromal vascular fraction; TEF: Tracheoesophageal fistula; TEP: Tracheoesophageal puncture; TMF: Tracheomedial fistula; VPI: Velopharyngeal insufficiency

**Acknowledgments**

Not applicable.

**Authors’ contributions**

Conceptualization, A.T., D.N., I.B., V.P., and G.T.; writing—original draft preparation, G.T. and G.D.R.; writing—review and editing A.T., D.N., S.B., and G.T. All authors have read and agreed to the published version of the manuscript.

**Funding**

This research was funded in part by the Italian Ministry of Health (Ricerca Corrente – IRECS IRE) and Istituto Regina Elena Cinque per Mille (5×1000 2015 to Gabriele Toietta). The funding sources had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

**Availability of data and materials**

Not applicable.
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing of interests.

Author details
1 Department of Surgical Science, University of Rome “La Sapienza”, Viale Regina Elena 324, 00161 Rome, Italy. 2 Department of General Thoracic Surgery, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Largo A. Gemelli 8, 00168 Rome, Italy. 3 Digestive Endoscopy Unit, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, 00168 Rome, Italy. 4 Department of Research, Advanced Diagnostic, and Technological Innovation, Translational Research Area, IRCCS Regina Elena National Cancer Institute, via E. Chianesi 53, 00144 Rome, Italy.

Received: 4 August 2020 Accepted: 7 October 2020
Published online: 23 October 2020

References
1. Hsu VM, Stransky CA, Bucky LP, Percec I. Fat grafting’s past, present, and future: why adipose tissue is emerging as a critical link to the advancement of regenerative medicine. Aesthet Surg J. 2012;32(7):892–9.
2. Mazzola RF, Mazzola IC. History of fat grafting: from rat fat to stem cells. Clin Plast Surg. 2015;42(2):147–53.
3. Simonacci F, Bertozzi N, Gioiaco MP, Grignaffini E, Rapiso E. Procedure, applications, and outcomes of autologous fat grafting. Ann Med Surg (Lond). 2017;20:49–60.
4. Trivisonno A, Rossi A, Monti M, Di Nunno D, Desouches C, Cannistra C, et al. Facial skin rejuvenation by autologous dermal microfat transfer in photoaged patients: clinical evaluation and skin surface digital profilometry analysis. J Plast Reconstr Aesthet Surg. 2017;70(8):1118–28.
5. Conde-Green A, Marano AA, Lee ES, Reisler T, Price LA, Milner SM, et al. Fat grafting and adipose-derived regenerative cells in burn wound healing and scarbing: a systematic review of the literature. Plast Reconstr Surg. 2016;137(1):302–12.
6. Trivisonno A, Abecasis M, Monti M, Toietta G, Bachir A. Adipose tissue: from energy reservoir to a source of cells for epithelial tissue engineering. In: Shifman MA, Di Giuseppe A, Bassetto F, editors. Stem cells in aesthetic surgery behind the surgery. Aesthet Surg J. 2016;36(4):488–96.
7. Vyas KS, Vazquez HC, Morrison S, Mogini B, Linton S, Hockensmith L, et al. Fat graft enrichment strategies: a systematic review. Plast Reconstr Surg. 2020;145(3):827–41.
8. Zelins ER, Brent EA, Longaker MT, Wan DC. Autologous fat grafting: the science behind the surgery. Aesthet Surg J. 2016;36(4):497–503.
9. Vyas KS, Vasconez HC, Morrison S, Mogini B, Linton S, Hockensmith L, et al. Fat graft enrichment strategies: a systematic review. Plast Reconstr Surg. 2020;145(3):827–41.
10. Zelins ER, Brent EA, Longaker MT, Wan DC. Autologous fat grafting: the science behind the surgery. Aesthet Surg J. 2016;36(4):488–96.

11. Bozza P, Majumdar AS. Adipose tissue-derived stromal vascular fraction in regenerative medicine: a brief review on biology and translation. Stem Cell Rev Rep. 2011;7(2):269–77.
12. Bortolotti L, Suuronen R, Miettinen S. The potential of adipose stem cells in regenerative medicine. Stem Cell Rev Rep. 2017;13(8):1451–68.
13. Lindroos B, Suuronen R, Miettinen S. The potential of adipose stem cells in regenerative medicine. Stem Cell Rev Rep. 2017;13(8):1451–68.
14. Zhuanathan S, Shi Y, Galipeau J, Krampera M, Leblanc K, Martin L, et al. Mesenchymal stem versus stromal cell: International Society for Cell & Gene Therapy (ISCT®) Mesenchymal Stromal Cell committee position statement on nomenclature. Cytotherapy. 2019;21(10):1019–24.
15. Gimble J, Guljak F. Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. Cytotherapy. 2003;5(3):362–9.
16. Caplan AI. Mesenchymal stem cells: time is changing the name! Stem Cells Transl Med. 2017;6(6):1445–51.
17. Baldari S, Di Rocco G, Piccoli M, Pozzobon M, Muraca M, Toietta G. Challenges and strategies for improving the regenerative effects of mesenchymal stromal cell-based therapies. Int J Mol Sci. 2017;18(10):2087.
18. von Bahr L, Batsis J, Moll G, Hägg M, Szakos A, Sundberg B, et al. Analysis of tissues following mesenchymal stromal cell therapy in humans indicates limited long-term engraftment and no ectopic tissue formation. Stem Cells. 2012;30(7):1575–85.
19. Rendra E, Scaccia E, Biack K. Recent advances in understanding mesenchymal stromal cells. F1000Res. 2019;8:Faculty Rev-156.
20. Jimenez-Puerta GJ, Marchal JA, Lopez-Ruz E, Gálvez-Martín P. Role of mesenchymal stromal cells as therapeutic agents: potential mechanisms of action and implications in their clinical use. J Clin Med. 2020;9(2):445.
21. Brown C, McKee C, Bakshi S, Walker K, Hakman E, Halassy S, et al. Mesenchymal stem cells: cell therapy and regeneration potential. J Tissue Eng Regen Med. 2019;13(9):1738–55.
22. Fan XL, Zhang Y, Li X, Fu QL. Mechanisms underlying the protective effects of mesenchymal stem cell-based therapy. Cell Mol Life Sci. 2020;77(14):2771–94.
23. Patrikioski M, Manorström B, Miettinen S. Perspectives for clinical translation of adipose/stromal stem cells. Stem Cells Int. 2019;2019:858247.
24. Choi S, Jeon BG, Chae G, Lee SJ. The clinical efficacy of stem cell therapy for complex peri-anal fistulas: a meta-analysis. Tech Coloproctol. 2019;23(5):411–27.
25. Carvello M, Lightner A, Yamamoto T, Kotze PG, Spinelli A, Mesenchymal stem cells for perianal Crohn’s disease. Cells. 2019;8(7):764.
26. Castro-Poceiro J, Fernández-Closter A, Panés J. Mesenchymal stromal cells in the treatment of peri-anal fistulas in Crohn’s disease. Immunotherapy. 2018;10(14):1203–17.
27. Panés J, García-Olmo D, Van Assche G, Colonbef IF, Reinsch W, Baumgart DC, et al. Long-term efficacy and safety of stem cell therapy (Cx601) for complex peri-anal fistulas in patients with Crohn’s disease. Gastroenterology. 2018;154(5):1334–42.e4.
28. Vermeulen BD, Siersema PD. Esophageal stenting in clinical practice: an overview. Curr Treat Options Gastroenterol. 2018;16(2):260–73.
29. Uelderman KV, Eaton J, Miuniappan A, Morse CR, Wright CD, Mathiesen DJ. Repair of large airway defects with bioprosthetic materials. J Thorac Cardiovasc Surg. 2016;152(5):1388–97.
30. Jain MK, Ramesh C, Sankar K, Lokesh Babu KT. Pedicled buccal fat pad in the management of oroantral fistula: a clinical study of 15 cases. Int J Oral Maxillofac Surg. 2012;41(8):1025–9.
31. Daif ET. Long-term effectiveness of the pedicled buccal fat pad in the closure of a large oroantral fistula. J Oral Maxillofac Surg. 2016;74(9):1718–22.
32. Eguedi P. Utilization of the buccal fat pad for closure of oro-antral and/or oro-nasal communications. J Maxillofac Surg. 1977;5(4):241–4.
33. Sapunzhiev NR, Nikiforova LT, Spassova BH, Ivanova D, Balev B. Endoscopic repair of pharyngocutaneous fistula following laryngectomy. Cureus. 2019;11(10):e5871.
34. Hespe GE, Alboonz CR, Mehrana BJ, Kraus D, Matros E. Case report: Pharyngocutaneous fistula closure using autologous fat grafting. Eplasty. 2013;13:e23.
35. Moreto M, Gabilondo J, Fernandez-Sanmiguel F. Treatment of a congenital esophageal fistula by injection of autologous fat. Endoscopy. 2014;46(Suppl 1):UCTN):E54–E5.
36. Díaz-Agero Álvarez P, García-Arranz M, Georgiev-Hristov T, García-Olmo D. A new bronchoscopic treatment of tracheomedastinal fistula using autologous adipose-derived stem cells. Thorax. 2008;63(4):374–6.
37. Aho JM, Dietz AB, Radel DJ, Butler GW, Thomas M, Nelson TJ, et al. Closure of a recurrent bronchopleural fistula using a matrix seeded with patient-derived mesenchymal stem cells. Stem Cells Transl Med. 2016;5(10):1375–S.
38. Petrella F, Spaggiari L, Acocella F, Barberis M, Bellomi M, Brizzola S, et al. Airway fistula closure after stem-cell infusion. N Engl J Med. 2015;372(1):96–7.
54. Rämö OJ, Salo JA, Isolauri J, Luostarinen M, Mattila SP. Tuberculous fistula of the bronchus. Thorac. Cardiovasc. Surg. 2001;49(7):374–8.

55. Burt M, Diehl W, Martini N, Bains MS, Ginsberg RJ, McCormack PM, et al. The surgical management of non-malignant aerodigestive tract fistulas. Eur. J. Cardiothorac. Surg. 1995;9(2):155–9.

56. Harley HR. Ulcerative tracheo-oesophageal fistula during treatment by irradiation. Br. Med. J. 1975;2(6011):738–9.

57. Qureshi YA, Muntzer Mughal M, Markar SR, Mohammadi B, George J, Périé S, Ming X, Dewolf E, St Guily JL. Autologous fat injection to treat trachoesophageal fistula over two decades. Arch. Surg. 1995;130(5):502–8 discussion 8-9.

58. Chakraborty R, Haag JB. Acquired aero digestive fistula in adults: case series and review. EC Pulmonol Respirer Med. 2019;8(2):129–39.

59. Parvin P, Obreja K, Sader R, Becker J, Schwarz F, Salti L. Surgical options in oroantral fistula management: a narrative review. Int J Implant Dent. 2018;4(1):10.

60. Kwon MS, Lee BS, Choi BJ, Lee JW, Ohe JY, Jung JH, et al. Closure of oroantral fistula: a review of local flap techniques. J Korean Assoc Oral Maxillofac Surg. 2020;46(1):58–65.

61. Busoni M, Deganello A, Gallo O. Pharyngocutaneous fistula following total laryngectomy: analysis of risk factors, prognosis and treatment modalities. Acta Otorhinolaryngol Ital. 2015;35(6):400–5.

62. Slater BJ, Rothenberg SS. Tracheoesophageal fistula. Semin Pediatr Surg. 1991;2(3):122–29.

63. Wang H, Ke M, Li W, Wang Z, Li H, Cong M, et al. Chinese expert consensus on diagnosis and management of acquired respiratory-digestive tract fistulas. Thorac. Cancer. 2018;9(11):1544–55.

64. Engum SA, Grosfeld JL, West KW, Rescorla FJ, Scherer LR. Analysis of treatment options and factors influencing morbidity and mortality in 227 cases of esophageal atresia and/or tracheoesophageal fistulae over two decades. Arch. Surg. 1995;130(5):502–8 discussion 8-9.

65. Gutiérrez Santamaria J, Masiá Gridilla J, Pamias Romero J, Giralt López-de-Segredo J, Bescós Atín MS. Fat grafting is a feasible technique for the sequelae of head and neck cancer treatment. J CranioMaxillofac Surg. 2017;45(1):93–8.

66. Nigh E, Rubio GA, Hallam J, Armstrong M, Debs L, Thaller SR. Autologous fat injection for treatment of velopharyngeal insufficiency. J Craniofac Surg. 2017;28(5):1248–54.

67. Contrera KJ, Tierney WS, Breyson PC. Autologous fat injection pharyngoplasty in adults with velopharyngeal insufficiency. Ann Otol Rhinol Laryngol. 2020;129(2):201–4.

68. Mattei A, Magalon J, Bertrand B, Girmaud F, Revis J, Viller M, et al. Autologous adipose-derived stromal vascular fraction and scarred vocal folds: first clinical case report. Stem Cell Res Ther. 2018;9(1):202.

69. Mattei A, Bertrand B, Joue E, Blaise T, Philandrianos C, Girmaud F, et al. Feasibility of first injection of autologous adipose tissue-derived stromal vascular fraction in human scarred vocal folds: a nonrandomized controlled trial. JAMA Otolaryngol Head Neck Surg. 2020;146(4):355–63.

70. Cantarella G, Mazzola RF, Gaffuri M, Iofrida E, Biondetti P, Forzenigo LV, et al. Structural fat grafting to improve outcomes of vocal folds’ fat augmentation: long-term results. Otolaryngol Head Neck Surg. 2018;158(1):135–43.

71. Cantarella G, Mazzola RF. Management of vocal fold scars by concurrent nanofat and microfat grafting. J Craniofac Surg. 2019;30(3):692–5.

72. Mikaelian DO, Lowry LD, Satoft RT. Lipoinjection for unilateral vocal cord paralysis. Laryngoscope. 1991;101(5):465–8.

73. Pagano R, Morsomme D, Camby S, Lejeune L, Finck C. Long-term results of 18 fat injections in unilateral vocal fold paralysis. J Voice. 2017;31(4):505 e1–9.

74. Lasso JM, Polletti D, Scola B, Gómez-Vilda P, García-Martin AI, Fernández-Santos ME. Injection laryngoplasty using autologous fat enriched with adipose-derived regenerative stem cells: a safe therapeutic option for the functional reconstruction of the glottal gap after unilateral vocal fold paralysis. Stem Cells Int. 2018;2018:8917913.

75. Laccourreye O, Crever-Buchman L, Le Pimpec-Barthes F, Garcia D, Riquet M, Brasinu D. Recovery of function after intracardial autologous fat injection for unilateral recurrent laryngeal nerve paralysis. J Laryngol Otol. 1998;112(11):1082–4.

76. Dayangku Norsuhazenah PS, Baki MM, Mohamad Yunus MR, Sabir Husin Athar PP, Abdullah S. Complications following tracheoesophageal puncture: a tertiary hospital experience. Ann Acad Med Singap 2010;39(7):565–566.

77. Grant N, Davison SP. Management of the post-tracheostomy scar. Laryngoscope. 2007;117(12):2107–9.

78. Borovikova AA, Ziegler ME, Baryard DA, Wirth GA, Paydar KZ, Evans GRD, et al. Adipose-derived tissue in the treatment of dermal fibrosis: antibiotic effects of adipose-derived stem cells. Ann Plast Surg. 2018;80(3):297–307.

79. Karmali RJ, Hanson SE, Nguyen AT, Skoracki RJ, Hansano MM. Outcomes following autologous fat grafting for oncologic head and neck reconstruction. Plast Reconstr Surg. 2018;142(3):711–80.

80. Rowan BJ, Lacayo EA, Sheng M, Anbalagan M, Gimble JM, Jones RK, et al. Human adipose tissue-derived stromal/stem cells promote migration and early metastasis of head and neck cancer xenografts. Aesthet Surg J. 2016;36(1):93–104.

81. Danan D, Lehmam CE, Mendez RE, Langford B, Koors PD, Dougherty MI, et al. Effect of adipose-derived stem cells on head and neck squamous cell carcinoma. Otolaryngol Head Neck Surg. 2018;158(5):882–8.

82. Mattei A, Magalon J, Bertrand B, Philandrianos C, Veran J, Giovanni A. Cell therapy and vocal fold scarring. Eur Ann Otorhinolaryngol Head Neck Dis. 2017;134(5):339–45.

83. Havas TE, Priestley KJ. Autologous fat injection laryngoplasty for unilateral vocal fold paralysis. ANZ J Surg. 2003;73(3):129–33.

84. McCulloch TM, Andrews BT, Hoffman HT, Graham SM, Karnell MP, Minnick C. Long-term follow-up of fat injection laryngoplasty for unilateral vocal cord paralysis. Laryngoscope. 2002;112(7 Pt 1):1123–8.

85. Han Y, Li X, Zhang Y, Chang F, Ding J. Mesenchymal stem cells for treatment of irradiated head and neck tissues by autologous fat transplantation. Plast Reconstr Surg. 2008;122(5):1626–34.

86. Dayan C, Ziegler ME, Ziegler PA, Baryard DA, Paydar KZ, Evans GRD, et al. Adipose-derived regenerative stem cells: a safe therapeutic option for the functional reconstruction of the glottal gap after unilateral vocal fold paralysis. Stem Cells Int. 2018;2018:8917913.
90. Petrella F, Spaggiari L. Repair of large airway defects with bioprosthesis. J Thorac Dis. 2017;9(10):3674–6.

91. Volpe BB, Santos Duarte AS, Ribeiro TB, Stocchero J, Kharramduyan P, Olalla Saad ST, et al. Mesenchymal stromal cells from adipose tissue attached to suture material enhance the closure of enterocutaneous fistulas in a rat model. Cytotherapy. 2014;16(12):1709–19.

92. Xue X, Yan Y, Ma Y, Yuan Y, Li C, Lang X, et al. Stem-cell therapy for esophageal anastomotic leakage by autografting stromal cells in fibrin scaffold. Stem Cells Transl Med. 2019;8(5):548–56.

93. Liu A, Zhang X, He H, Zhou L, Naito Y, Sugita S, et al. Therapeutic potential of adipose tissue for cell- and tissue-based therapies: concise review. Stem Cells Transl Med. 2019;8(1):125–31.

94. Lightner AL. The present state and future direction of regenerative medicine for perianal Crohn’s disease. Gastroenterology. 2019;156(8):2208–16.e1.

95. Garcia-Olmo D, Garcia-Arranz M, Garcia-LG, Cuellar ES, Blanco IF, Prianes LA, et al. Autologous adipose-derived stromal and vascular cells by overexpression of manganese peroxiredoxin promote survival and engraftment of transplanted adipose tissue for cell- and tissue-based therapies: concise review. Stem Cells Transl Med. 2019;8(12):1265–71.

96. Gassler M, Lobato RC, Díaz JM, Singh K, Verpaele A, Tonnard P. Expanding the clinical applicability of extracellular vesicles: a review. Stem Cell Res Ther. 2020;11(1):312.

97. García-Olmo D, García-Arranz M, García-LG, Cuellar ES, Blanco IF, Prianes LA, et al. Autologous stem cell transplantation for treatment of rectovaginal fistula in perianal Crohn’s disease: a new cell-based therapy. Int J Color Dis. 2020;35(5):451–4.

98. Garcia-Arranz M, Garcia-Olmo D, Herreros MD, Gracia-Solana J, Guadalajara H, de la Portilla F, et al. Autologous adipose-derived stem cells for the treatment of complex cryptoglandular perianal fistula: a randomized clinical trial with long-term follow-up. Stem Cells Transl. Med. 2020;9(3):295–301.

99. Raposo E, Ciliberti R. Clinical use of adipose-derived stem cells: European legislative issues. Ann Med Surg (Lond). 2017;24:61–4.

100. Alexander RW. Biocellular regenerative medicine: use of adipose-derived secretome and vesicles for lung actuated delivery strategy. ACS Nano. 2018;12(10):9800–14.

101. Laureti S, Gionchetti P, Cappelli A, Vittori L, Contedini F, Rizzello F, et al. Stem Cell Research & Therapy. 2020;20(2):12531.

102. Baldari S, Di Rocco G, Gentile P, et al. Intraoperative strategies for minimal manipulation of autologous stem/stromal cells and its clinical indications of mechanically isolated stromal vascular fraction: a systematic review. Stem Cell Research & Therapy. 2020;11(2):131.

103. Alexander RW, Baldari S, Cohen SR, Di Rocco G, Gentile P, et al. Stem Cell Research & Therapy. 2020;11(3):368.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.