



## REVIEW ARTICLE

## Tissue engineering and regenerative medicine in otorhinolaryngology

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## Abstract:

**Background and Aim:** Regenerative medicine has been gaining popularity in the field of medicine, and the possibilities for tissue regeneration are immense in the field of otorhinolaryngology, which involves sensory organs and vital functions such as breathing and swallowing. Regenerative strategies offer the potential to restore functions such as hearing, facial expression, olfaction, and speaking, thereby reducing the disadvantages and risks related to traditional reconstruction strategies. This review summarizes the progress of regenerative medicine in otology and hearing, laryngeal surgery, rhinology, and craniofacial reconstruction.

**Relevance for Patients:** Patients can be informed about the progress of regenerative medicine in the field of otorhinolaryngology and how it has evolved to ameliorate the symptoms of common diseases or cure even more severe ones.

## 1. Introduction

Over the past decade, regenerative medicine and tissue engineering have gained popularity in the medical and pharmaceutical sectors, owing to the ability of tissue engineering to correct medical defects using scaffolds that mimic the natural form and function of many organs and tissues. Regenerative medicine can also be applied in the field of otorhinolaryngology to treat common anatomical and physiological deficiencies. These deficiencies are congenital by nature or can develop from head and neck cancer treatment (e.g., radiotherapy and surgery). Tissue replacement, using grafts derived from other tissues and artificial materials, is a traditional approach to addressing these deficiencies [1].

Cells, biocompatible scaffolds, and bioactive factors are the three major types of treatment used in regenerative medicine (Table 1) [2,3]. Cells, including stem, progenitor, or differentiated cells, can be employed to rebuild tissues and alter the immune response and cell behavior [4]. Scaffolds, synthetic or biological, are 3D structures designed to fit into defects and restore the diseased organs' function. They can be combined with bioactive factors or cell components to control differentiation and migration to specified tissues [5]. *In vivo*, the cell processes involved in cell regeneration can be influenced by various molecules, including growth factors, cytokines, hormones, and other compounds [6,7]. In addition, these strategies have disadvantages, such as the requirement of immunosuppressive drugs, donor site morbidity, infection, and rejection risks [8]. Whilst the transplantation of bioengineered tissues can overcome the latter hindering factors, extensive trials to overcome the bio-ethical challenges are necessary to significantly improve the use of regenerative tissue medicine in the field of otorhinolaryngology.

**Table 1.** Tissue engineering and regenerative medicine concepts

Stem cells			Bioactive factors	Scaffolds	
Totipotent stem cells	Pluripotent stem cells	Multipotent stem cells		Biological material	Synthetic material
Differentiate into embryonic and extra-embryonic tissues	Differentiate into the entire range of derivatives of all three embryonic germ layers	Differentiate into derivatives of multiple cell lineages	Cytokines, growth factors, hormones, and morphogenic proteins	Trachea and aortic allograft	Ceramic, metal, polymer, and composite
Obtained from an early embryo in the two-cell stage	Includes ESC and iPSC	Includes NSCs and MSCs from the bone marrow, fat, skin, umbilical cord, and/or muscle			

Abbreviations: ESC: Embryonic stem cell; NSCs: Neural stem cells; MSCs: Mesenchymal stem cells; iPSC: induced pluripotent stem cell.

The field of otorhinolaryngology is extensive, including many different tissues and functions such as hearing, balance, olfaction, facial expression, breathing, and speaking. The diverse cells and tissues in the ear, nose, and throat, which are responsible for the related vital functions, contribute to the heterogeneity challenging the exploitation of regenerative therapies. This review aims to narratively summarize the current state of tissue engineering and regenerative medicine in otorhinolaryngology.

## 2. Methods

This paper provides a literature overview of regenerative medicine and tissue engineering in otorhinolaryngology. A literature search was conducted in PubMed, CINAHL, and Scopus using the following terms: “regenerative medicine,” “tissue engineering,” “regenerative surgery,” “stem cells,” “ear,” “cochlea,” “hearing loss,” “nose,” “larynx,” “head and neck,” “vocal fold,” “trachea,” “craniofacial,” “otology,” “rhinology,” “laryngology,” “salivary glands,” and “otorhinolaryngology,” from database inception to 2022. We used Boolean operators to refine our search. In addition, review articles from the reference list were included.

All randomized and non-randomized controlled prospective and retrospective trials and case series of two or more patients of any age were included. Only articles written in English were further considered for the study. Duplicates were excluded. Articles related to oral surgery and maxillofacial surgery were excluded. Although randomized controlled trials were prioritized and human clinical trials were preferred, animal trials were also reviewed and included. Most articles were pre-clinical animal studies, reflecting the current state of research on this topic. Information from completed early-phase clinical trials was included, while ongoing clinical trials were excluded. Articles were analyzed and selected based on relevance to the topic of interest.

## 3. Results

The literature search identified 622 studies, of which 105 fit the eligibility criteria for data curation. Forty studies reported on otology and hearing, eight on craniofacial cartilaginous reconstruction, four on salivary glands, and 46 on laryngology.

## 4. Applications in Otology and Hearing

### 4.1. Cochlea

Loss of hearing, congenital or acquired, results in the hair cells' inability to function correctly and subsequent death [9]. The absence of hair cells causes the death of spiral ganglion neurons (SGNs) [9]. The mature mammalian cochlea in mammals is incapable of hair cell regeneration [10]. Intracellular stem cell activation and external stem cell transplantation are two approaches in regenerative medicine that can potentially treat sensorineural hearing loss. The first approach is to stimulate the stem cells present in the organ of Corti, leading to the replacement of damaged hair cells. The second technique involves introducing stem cells from an external source into the inner ear. Several papers reported using mesenchymal stem cells (MSCs) in animal models with hearing loss (Table 2). The injection of primary MSCs into the cochlea may result in the survival of hair cells [9]. However, there is currently minimal indication of the transdifferentiation of MSCs into hair-like cells or neurons *in vivo* [9].

Jang *et al.* implanted human bone marrow MSCs (BM-MSCs) into the cochlea of neomycin-deafened guinea pigs, resulting in a more significant number of SGNs as compared to the control group [11]. MSCs have also been utilized in human research but with minimal hearing enhancement [12]. In 2015, a clinical experiment explored the efficacy of transplanting autologous BM-MSCs in patients with sensorineural hearing loss. Two individuals were intravenously injected with cells, but their hearing did not improve [12]. Eleven children participated in another clinical trial using an autologous umbilical cord stem cell infusion [13]. There were no adverse events reported, and significant improvement in hearing was discovered from several hearing tests.

It is more difficult to regenerate the auditory nerve using stem cell technology due to the electrical features of those cells and the requirement for an adequate connection with the remaining residual auditory neurons [14]. *In vitro* studies have revealed that MSCs obtained from the olfactory mucosa can stimulate the myelination of oligodendrocytes [9]. However, an ideal transplantation method has not been established thus far. Systemic injection, injection into the scala tympani via the round window or a basal turn cochleostomy, and injection into the scala media are some of the stem cell transfer methods for the cochlea [14].

**Table 2.** Animal model studies on MSCs in hearing loss

Study	Cell type	Method	Model	Results
Pandit et al., 2011 [20]	Human OSCs	Injection of OSCs into the cochlea through lateral cochleostomy	Mice	The hearing thresholds were substantially lower in the experimental group as compared to the control group. There was no integration of transplanted cells into cochlear tissues.
Choi et al., 2012 [21]	Human BM-MSCs	Intravenous injection of MSCs	Rats	Most of the injected MSCs were located in the lungs and a small number were in the spiral ganglion region.
Kasagi et al., 2013 [22]	Mouse MSCs	Infusion of MSCs into the ampulla of SCC	Mice	MSCs moved to the cochlea and differentiated into fibrocytes. The auditory brainstem response (ABR) did not alter in the experimental group compared to the control group.
Bas et al., 2014 [23]	Human olfactory MSC-like stem cells	Applied MSCs to cochlear cultures	Rats	The spiral ganglion neuron population was restored.
Jang et al., 2015 [11]	Human bone marrow neural-induced MSCs	hMSCs were transplanted into the scala tympani of damaged cochlea	Guinea pigs	Transplanted hMSCs were found within the perilymphatic space, the organ of Corti, along the cochlear nerve fibers, and in the spiral ganglion. The quantity of SGNs was elevated in comparison to the control group.
Yoo et al., 2015 [24]	Human adipose tissue-derived MSCs	Intraperitoneal injection of hMSCs	Mouse model of autoimmune hearing loss	There were improvements in hearing function in the intervention group as compared to the control group.
Xu et al., 2016 [25]	Rat olfactory epithelium neural stem cells	Stem cells were injected straight into the cochlea	Rats	There was migration of stem cells around the SGNs, and hearing loss improved as determined by ABR.
Le et al., 2017 [26]	Magnetically labeled rat MSCs	MSCs were injected into the systemic circulation	Rats	The presence of MSCs in the cochlea was identified, and the experimental group had a significant increase in hearing threshold levels.
Chen et al., 2018 [27]	Human urinary cells reprogrammed into iPSCs	OEPs were produced from iPSCs and transplanted into the cochlea of mice	Mice	A healthy donor's urine cells were converted into iPSCs. These were stimulated to develop into OEPs and hair cell-like cells. Co-cultured hair cell-like cells generated from OEP developed synaptic connections with SGNs in vitro. OEPs were produced from iPSCs and transplanted into the cochlea of mice. Some transplanted cells moved in the organ of Corti to the site of resident hair cells, developed into hair cell-like cells, and established synaptic connections <i>in vivo</i> with the native SGNs.
Betini et al., 2018 [28]	Human BM-MSCs ASCs	MSCs were intravenously injected into deafened mice	Mice	Both types of MSCs induced the regeneration of damaged sensory cochlear cells.
Mittal et al., 2019 [29]	Rat BM- MSCs	Transtympanic delivery of BM MSCs	Rats	There were ABR and DPOAE, and the cochlear function of the treated animals normalized as compared to the control groups. No inflammatory reactions were detected.
Abd El Raouf et al., 2019[30]	Harderian gland-derived stem cells (HG-SCs)	Intravenous injection of HG-SCs	Guinea pigs	In the HG-SC-treated group, both cochlear structure and functions were restored, along with a considerable increase in hair cell numbers, spiral ganglionic cell count, and stria vascularis thickness to levels comparable to those of the control group.
Radeloff et al., 2021 [31]	ASCs	ASCs were autologously transplanted into the scala tympani before the insertion of a cochlear implant on one side	Guinea pigs	ASC transplantation enhanced the number of SGNs as well as their peripheral neurites. Mean ABR thresholds were lower, and suprathreshold amplitudes were greater in ASC-transplanted mice, indicating a bigger population of auditory nerve fibers.

Abbreviations: ABR: Auditory brainstem response; ASC: Adipose tissue-derived stem cell; BM-MSC: Bone marrow mesenchymal stem cell; DPOAE: Distortion product otoacoustic emissions; HG-SCs: Harderian gland-derived stem cells; hMSCs: human mesenchymal stem cells; iPSCs: induced pluripotent stem cells; MSC: Mesenchymal stem cell; OEPs: Otic epithelial progenitors; OSC: Olfactory stem cell; SCC: Superior semicircular canal; SGNs: Spiral ganglion neurons.

Furthermore, the cells must be able to incorporate into the organ of Corti following injection and survive the potentially lethal high potassium concentration of the endolymph [15,16]. Lee et al. evaluated the viability of human embryonic stem cells (ESCs) in the cochlea of deaf guinea pigs preconditioned to have low potassium levels [17]. Their study indicated that temporarily lowering the potassium concentration in the endolymph before transplantation, by flushing it with sodium caprate, contributed to a 1-week survival of human ESCs in the endolymph.

Injecting genes and medications to rejuvenate the present cells of the inner ear is challenging. Researchers have already tested the efficacy of the gene-editing technology CRISPR/Cas9 in treating animal models with autosomal dominant hearing loss [18]. To activate the existing cells, the future treatment will involve combining stem cell therapy, gene therapy, and pharmacological therapy. In 2020, Huang et al. developed an induced pluripotent stem cell (iPSC) line from a 7-year-old male patient with a homozygous *GJB2* c.235delC mutation [19]. Human SOX2, OCT4, KLF4, and c-MYC reprogramming factors were expressed in reprogrammed peripheral blood mononuclear cells. Five iPSC clones were manually selected, grown, and stored; their capacity to differentiate into three germ layers was revealed. Genetic technology can precisely regulate stem cells *in vivo*, ameliorating their applicability in therapies.

#### 4.2. Tympanic membrane (TM)

The TM is a thin membrane between the external and middle ear. TM perforations (TMPs) are a significant issue in otology. While acute TMPs can heal naturally, chronic TMPs require surgery (i.e., tympanoplasty). The standard surgical procedure is performed through tympanoplasty, using the perichondrium or temporalis fascia to rectify the TMP. In regenerative therapy, a range of scaffold materials (e.g., hyaluronic acid [HA], collagen, chitosan, and gel foam), growth factors, and cells have been used as therapies for TMP (Table 3).

#### 4.3. Growth factors in TMP regeneration

Growth factors have been studied for the repair of TMPs. A randomized controlled trial conducted by Lou and Lou included 184 patients with traumatic TMP [32]. The intervention groups received drops containing EGF, FGF-2, and ofloxacin, respectively [32]. The study reported that all treatment groups had significantly shorter closure times than the control group. A randomized controlled trial with 93 study subjects treated with basic fibroblast growth factor (bFGF) displayed a substantially higher closure rate and a considerably shorter closure time in the experimental group than in the control group [33]. Cai et al. examined the short- and long-term detrimental effects of fibroblast growth factor-2 (FGF-2) therapy in 134 patients with tympanic perforations. The results revealed that the total closure rate and the closure healing duration were much better in the FGF-2 group [34]. Kanemaru et al. investigated the use of fibrin glue and gelatin sponge with bFGF, the use of which demonstrated increased healing rates of complete TMP closure as compared to the control group [35].

#### 4.4. Stem cells in TM regeneration

The use of stem cells in regenerative techniques for TMP recovery has been studied in animal models [36]. Scaffold materials can be used as supporting structures to provide mechanical assistance and deliver cells for cell proliferation and differentiation. Combining scaffolds with MSCs or growth factors has improved TPM healing efficacy. In two studies by Goncalves et al., the combination of BM-MSCs with a HA scaffold or gelatin sponge, respectively, resulted in enhanced TMP recovery [37,38].

In a clinical trial by Vozel et al., [11] patients were given autologous platelet- and extracellular vesicle-rich plasma as a therapy for persistent postoperative inflammation of the temporal bone cavity. A persistent postoperative inflammation of the temporal bone cavity is defined as a chronically discharging radical mastoid cavity that is oftentimes the result of a canal wall-down mastoidectomy. The findings of the trial indicated the remarkable efficacy of autologous platelet- and extracellular vesicle-rich plasma in treating persistent postoperative inflammation of the temporal bone cavity, thereby suggesting its promising use after conventional surgical and conservative therapies have been exhausted [39].

#### 4.5. Ossicles

Through tissue engineering, ossicle reconstruction is performed by cultivating MSCs on bioresorbable 3D scaffolds. Danti et al. developed partial ossicular replacement prosthesis (PORP)-like scaffolds with a biocompatible and biodegradable polymer [47]. The poral characteristics were analyzed using micro-CT, and the capacity to support human MSC (hMSC) colonization and osteoblastic development *in vitro* was analyzed quantitatively and qualitatively [48]. The findings demonstrated that the poral characteristics of PORP-shaped scaffolds were necessary to support the colonization of hMSCs and their osteoblastic maturation *in vitro*.

#### 4.6. Cartilaginous craniofacial components

Autologous cartilage is the gold standard for nasal and auricular reconstruction. However, the use of allogenic and synthetic materials for the cartilage is known to increase the risk of tissue rejection, resorption, extrusion, and infection. In this regard, regenerative engineering methods may be preferred as the engineered cartilages closely resemble native cartilages and can be produced in large amounts. In addition, these cartilages can be specifically shaped by harvesting cartilage cells from the auricle or septum and growing them in a specific 3D scaffold. Likewise, growth factors can facilitate the growth and differentiation of the cartilage cells in the scaffold.

In 2004, autologous cultured chondrocytes were utilized in human nose reconstruction for the first time [49]. Yanaga et al. extracted the chondrocytes from the conchal cartilage and cultivated them *in vitro*. The chondrocytes were then injected into a subcutaneous pocket above the nasal bone. No complications were reported after a 2-year follow-up period. In 2009, Yanaga et al.



**Table 3.** Clinical trials of TMP treatment with MSCs, scaffolds, and growth factors

Author	Number of patients/study model	Treatment	Outcome
Raj <i>et al.</i> , 2011 [40]	42 patients; two groups: 21 patients per group	Tympanoplasty type 1 with acellular dermis was performed in one group, and type 1 tympanoplasty with temporal fascia was performed in the other group (control).	There were no significant differences in terms of the graft success rate and hearing improvement. However, the acellular dermis had a shorter operative time and lesser postoperative pain.
Kanemaru <i>et al.</i> , 2011 [35]	63 patients; two groups: 53 were assigned to the bFGF group and 10 were assigned to the control group	Fibrin glue and gelatin sponge with bFGF were used in the bFGF group.	There were significantly greater rates of TMP closure in the bFGF group.
Roosli <i>et al.</i> , 2011 [41]	20 patients; two groups: 10 in the placebo group and 10 in the intervention group	Topical PDGF application on TMPs in the intervention group.	There were no significant differences in terms of success rate (reduction of perforation size by 50% or more) and hearing thresholds between the two groups.
Lou <i>et al.</i> , 2012 [42]	94 patients; three groups: (1) direct FGF application, (2) FGF via Gelfoam, and (3) control group	Topical FGF, Gelfoam with FGF.	The closure rates in the FGF-treated groups were significantly increased as compared to the control group, but there was no difference in closure rates in patients who received FGF directly and those who received FGF through Gelfoam.
Lou and Wang, 2015 [33]	93 patients; two groups: randomized into control and bFGF-treated groups	Topical bFGF application.	There were significantly higher rates of closure and shorter closure times in the bFGF-treated group than in the control group.
Lou <i>et al.</i> , 2016 [43]	86 patients; three groups:(1) EGF, (2) bFGF, and (3) control group	Topical bFGF and EGF application.	There was no substantial difference in the closure rates and closure times between the bFGF, EGF, and control groups.
Lou <i>et al.</i> , 2016 [44]	97 patients; two groups: topical application of EGF in one group and a control group	Topical EGF application.	The total closure rates did not significantly differ between the two groups. The total average closure time in the control group was significantly longer than in the EGF group.
Lou and Lou, 2017 [32]	184 patients; four groups: (1) EGF treatment, (2) FGF-2 treatment, (3) 0.3% ofloxacin drops treatment, and (4) control group	EGF, FGF-2, and ofloxacin drops 0.3% were applied in the three treatment groups, respectively.	The three treatment groups exhibited significantly shorter closure times as compared to the control group. Neither the closure rate nor closure time differed significantly among the three treatment groups.
Zheng Cai <i>et al.</i> , 2018 [34]	134 patients; two groups: randomly divided into a control group and an FGF-2 treatment group	FGF-2 application on TMP in the treatment group.	The overall closure rate was significantly different between the FGF-2 treatment group and the control group. The FGF treatment group had a considerably shorter closure time than the control group.
Kanemaru <i>et al.</i> , 2021 [45]	20 patients; non-randomized, single-arm study	A gelatin sponge with bFGF and fibrin glue was applied.	At 16 weeks, complete closure of the TMP was observed in 15 of 20 patients, and the ratio of hearing improvement and air-bone gap was 100%.
Lou <i>et al.</i> , 2021 [46]	29 patients; two groups: 13 in the bFGF alone group and 16 in the myringoplasty group	bFGF application in one group.	It was indicated that bFGF alone facilitated the repair of chronic and small TMPs but was ineffective for medium-sized TMPs.

Abbreviations: bFGF: basic fibroblast growth factor; EGF: Epidermal growth factor; FGF: Fibroblast growth factor; MSC: Mesenchymal stem cell; PDGF: Platelet-derived growth factor; TM: Tympanic membrane; TMP: Tympanic membrane perforation.

harvested chondrocytes from the auricular cartilage and created a gelatinous chondroid matrix, which was then injected into the nasal dorsa of 75 patients [50]. The gel hardened to form a neo-cartilage within a few weeks, and the cartilages were still functional after 6 years. Autologous nasal septal chondrocytes were used in human exploratory trials in 2014 for nasal alar reconstruction. The cells were cultivated on collagen membranes for 4 weeks before being

used for nose restoration in five patients. The patients did not report any complications in the subsequent twelve months and were pleased with the aesthetics and functionality of the reconstructed nose [51]. In 2018, Zhou *et al.* designed a specific scaffold based on a healthy ear for auricular reconstruction in five patients. The scaffold was composed of biodegradable polymers cultivated with autologous chondrocytes. The results were satisfactory, and the

follow-up lasted for 2.5 years without any incidents of deformation being reported [52]. In 2018, a 7-year-old patient with arrhinia underwent nasal reconstruction using a 3D-printed nasal stent to prevent nasal cavity constriction. In this clinical research, a custom-made silicone nasal stent was manufactured utilizing 3D printing technology. The muco-epithelial tissue successfully regenerated within 2 months following the stent placement. External nose shape, nasal passage structure, and respiratory functions were in good condition after 3 years following the stent removal without additional medical intervention [53].

The most prominent use of auricular cartilage was in 1997 when polyglycolic acid scaffolds were shaped into the 3D structure of a human ear, seeded with bovine chondrocytes, and transplanted into the dorsal pockets of mice [54]. Thereafter, research was carried out in regenerative medicine with synthetic scaffolds and cultured chondrocytes. Yanaga *et al.* injected a combination of autologous serum and autologous chondrocytes from the outer ear cartilage into the ear, nose, and chin of 32 patients with craniofacial or nasal abnormalities [49]. A two-stage transplantation procedure was performed for auricular and nasal/chin reconstructions, respectively [55,56]. In the former, chondrocytes were first extracted from the residual auricular cartilage of four children with microtia and cultivated respectively into a subcutaneous pocket of fascia in the lower abdomen for 4 weeks. Subsequently, the children did not report any adverse events during the 2- to 5-year follow-ups [55]. Similarly, 18 individuals were treated with a comparable nasal/chin reconstructive technique [56].

#### 4.7. Salivary glands

The glands of the upper aerodigestive tract (i.e., parotid, sublingual, and submandibular glands) and minor salivary glands are known to produce saliva [57]. Hypofunctional can be caused by radiation therapy for head and neck cancer, Sjogren's syndrome (SS), and various medications. Possible oral problems caused by hyposalivation include mucosal infections, dysphagia, and aspiration pneumonia. In animal models, BM-MSCs have demonstrated the therapeutic capability to rebuild the salivary glands [58,59]. Xu *et al.* reported a successful restoration of the secretory function of salivary glands in animal models and SS patients using MSC therapy [58]. Adipose tissue-derived stem cells (ASCs) have also been studied in clinical trials on irradiation-induced hypofunctional salivary glands in patients [60]. The phase I/II clinical trial evaluated the efficacy and safety of ASC-based cell therapy, whereby the submandibular glands were injected with autologous ASCs. In the ACS-treated group, the unstimulated total salivary flow rate (assessed after 1 and 4 months) was significantly more than the baseline (pre-treatment). In contrast, the placebo group reported a decrease in salivary flow rate after 1 month and a less prominent increase after 4 months.

## 5. Applications in Laryngology

### 5.1. Vocal folds

Lamina propria (LP) is a flexible, collagen- and elastin-rich vibratory connective tissue layer between the epithelium and the

muscular tissue of the vocal cords. Trauma, surgery, or long-term vocal misuse are the main causes of scar formation, leading to loss of pliability of the vocal folds. LP scarring causes stiffness and reduces viscosity that changes the tissue biomechanics of the vocal fold [61]. As a result, the normal mucosa wave during phonation is disrupted, thereby affecting the vocals [62,63]. Nonetheless, damaged vocal folds can be regenerated through several methods, including cell therapy, developing and implementing a scaffold, and using growth factors.

### 5.2. Cell therapy

Fibroblasts and stem cells are extensively studied for vocal fold regeneration. Fibroblasts produce a large proportion of the extracellular matrix (ECM) in the LP and further support the LP. Fibroblasts resemble MSCs because they possess the same cell surface markers and differentiation capacity [63]. Chhetri *et al.* were the first to study the use of autologous fibroblasts from the buccal mucosa. In this study, fibroblasts were injected into the LP of a canine model, subsequently improving the vocal fold mucosal waves and acoustic characteristics. In addition, histological assessments revealed increased fibroblasts, collagen, and reticulin and decreased elastin [64]. Chhetri *et al.* also conducted a pilot study where five individuals with damaged vocal folds were injected with autologous fibroblasts from the buccal mucosa [65]. Four out of five patients demonstrated subjective and objective improvements in the vocal quality and mucosal wave. In another study, Ma *et al.* examined the efficacy of fibroblasts in 15 patients with vocal fold scarring or atrophy using postauricular skin-derived autologous fibroblasts and reported improvements in the mucosal wave without any side effects [66].

Likewise, BM-MSCs have demonstrated positive indications in animal studies [67,68]. In 2020, a phase I/II human clinical trial was conducted with 16 patients to investigate the treatment of vocal fold scarring with autologous BM-MSCs [69]. The patients were followed up for a year, and two-thirds of the participants demonstrated improvements in vocal vibration and flexibility.

There was another phase I/IIA clinical trial that demonstrated the efficacy and safety of adipose-derived regenerative cell-enriched fat grafting to repair glottal gaps following unilateral vocal fold paralysis [70].

### 5.3. Bioactive factors

Hirano *et al.* studied the use of bFGF in treating atrophic human vocal fold. One week after the bFGF injection, the aerodynamic and acoustic parameters displayed improvements that lasted for 3 months [71]. In a recent study by Hirano *et al.*, local injections of bFGF were performed in 100 cases of vocal fold disease [72]. The findings of the study indicated that intracordal injection of bFGF resulted in voice improvements without any significant adverse effects. Hirano *et al.* also reported that bFGF enhanced the synthesis of HA in fibroblasts and decreased collagen deposition in the vocal folds of aged rats [73]. Subsequently, Hirano *et al.* conducted a clinical trial with 10 patients having aged vocal folds with scar tissue and sulcus vocalis [74]. The findings of the

trial reported that all patients displayed improvements in speech function and acoustic and aerodynamic measurements.

Hepatocyte growth factor (HGF) is another growth factor that regulates cell proliferation and differentiation and is commonly associated with vocal fold recovery. HGF has an antifibrotic effect and can boost HA levels, reduce collagen production, and stimulate cell growth and migration [75]. In the event of an injury to the vocal folds, HGF can be found in the LP and the epithelium, and its angiogenic and antifibrotic properties could facilitate wound healing of the vocal folds [76]. In one study, it was reported that HGF prevented excessive collagen deposition and restored the levels of elastin, collagen, or HA to normal levels relative to an uninjured vocal fold [77]. Hirano *et al.* also conducted a clinical trial involving 18 individuals with vocal fold scarring or sulcus, and the findings of the trial indicated an improvement in voice measurements [78].

#### 5.4. Vocal fold scaffolds

Several types of scaffolds have been developed for 3D LP replacement, including biological polymers, decellularized organ matrices, synthetic biomimetic hydrogels, and synthetic polymers [79]. These scaffolds can be either injected or attached during surgery, wherein the scaffolds have biomechanical similarities with the LP, transport cells, and other bioactive components. Imaizumi *et al.* successfully employed biodegradable gelatin hydrogel microspheres as a delivery vehicle for bFGF in a rabbit model with vocal fold damage, and the larynxes reportedly displayed better vibratory function and reduced scarring based on histological assessments [80]. HA-modified hydrogel scaffolds have reportedly promoted fibroblast spreading, proliferation, and collagen/glycosaminoglycan production [81]. Likewise, acellular biological scaffolds have similar biological composition and architecture as native tissues. Therefore, the biological scaffolds can facilitate host cell adhesion, motility, and infiltration and secrete pro-angiogenic growth factors [82].

#### 5.5. Larynx

The larynx is composed of multiple tissue types, which presents as a challenge when restoring its function and structure. Patients with an advanced primary tumor have limited treatment options. Total or partial laryngectomy (removal of part or all of the larynx) remains the primary treatment method for advanced primary tumor, but this would lead to speech, breathing, and swallowing deficiencies. Nonetheless, bioengineered laryngeal structures have been developed and tested in animal and human models.

Animal studies have demonstrated that cartilage-like grafts may be effectively employed for partial laryngeal cartilage replacement when stem cells are grown in the scaffolds [48,83]. In contrast, aortic allografts have been utilized to repair hemilaryngeal abnormalities in human studies [84,85]. Brookes *et al.* conducted the first animal study demonstrating that primary skeletal muscle progenitor cells and standardized oligomeric collagen may be used to generate functioning, 3D tissue-engineered skeletal muscle [86].

The human larynx is typically decellularized to effectively produce scaffolds. Baiguera *et al.* evaluated the effectiveness of the modified detergent-enzyme method (DEM) as a decellularization technique for creating human laryngeal acellular matrices that are structurally and mechanically comparable to the original larynx [87]. Twenty-five cycles of DEM created a bioengineered human laryngeal matrix that was physically and mechanically identical to the biological larynx with pro-angiogenic factors. Al-Qurayshi *et al.* evaluated the effect of DEM on the larynx and cricoarytenoid joint of human cadavers. In this study, five fresh frozen human cadaveric larynxes were effectively decellularized, as evidenced by the considerable DNA depletion and ECM preservation, to regenerate a non-immunogenic larynx from a biological scaffold [88]. However, the use of numerous detergents and enzymes in DEM weakened the cricoarytenoid joints. Moser *et al.* recently described the synthesis of laryngeal grafts utilizing decellularized canine laryngeal scaffolds that were recellularized with primary human cells, and this provided the foundation for developing functional laryngeal scaffolds with composite tissue grafts [89]. In another study, Huber *et al.* used a porcine-derived ECM to reconstruct the larynx in mature dogs, demonstrating the constructive remodeling of a xenogeneic acellular biological scaffold material [90]. Thyroid cartilage and thyroarytenoid muscle were restored, and histologic investigation revealed glandular structures, a complete epithelial lining, cartilaginous structures, and skeletal muscle tissue in the reconstructed tissue. The microstructure and macrostructure of the recreated tissue were nearly the same as the original. Porcine laryngeal scaffolds were decellularized and subsequently seeded with human BM-MSCs in two recent studies on laryngeal replacement [91,92]. Both of the studies featured the decellularization of the whole larynx and the production of a safe and biocompatible biological scaffold with the ability to stimulate re-epithelialization and submucosal development. More importantly, the implanted scaffolds supported normal respiratory functions, in addition to proper swallowing and vocalization. The aforementioned studies and their promising results have established the prospect of successful functional partial laryngectomy reconstruction and total laryngeal regeneration.

#### 5.6. Trachea

Patients with congenital malformations or acquired tracheal stenosis after trauma or malignancy are candidates for reconstruction as other minor defects can be easily managed with tracheal resection and end-to-end anastomosis. Airway reconstruction requires a combination of scaffolds seeded with cells. In particular, two studies involving children utilized decellularized deceased donor trachea [2,93,94]. In the first study, the decellularized cadaveric donor tracheal scaffolds were planted with BM-MSCs and autologous epithelium before transplantation in a 12-year-old child suffering from congenital stenosis of the trachea. The child was topically applied with human recombinant erythropoietin to stimulate angiogenesis and transforming growth factor to promote chondrogenesis. At the 2-year follow-up, the child had a functioning airway and had

returned to school [2]. Subsequently, the 4-year follow-up study reportedly validated the long-term viability of a decellularized tissue-engineered trachea within the child [93]. In the second study, a 15-year-old girl with severe tracheal stenosis was treated with a tissue-engineered decellularized tracheal graft seeded with stem cells [94]. A decellularized tracheal allograft, seeded with autologous respiratory epithelial cells and MSCs, was applied. Early findings were promising, but a critical incident speculated as an intrathoracic hemorrhage, resulted in rapid airway blockage and her subsequent death 3 weeks after the transplantation.

In addition, synthetic scaffolds have been utilized for tracheal restoration. Omori *et al.* were the first to use regenerative procedures to restore the trachea of a thyroid cancer patient [95]. A polypropylene mesh tube coated with a collagen sponge was utilized as a tissue scaffold. The process included right hemithyroidectomy, trachea resection, and scaffold-assisted tracheoplasty. The right side of the three trachea ring segments was removed, and the trachea defect was bridged by suturing the scaffold material. In 2008, Omori *et al.* also utilized similar synthetic implants in four patients to successfully repair their larynx and/or trachea [96].

In animal studies involving aortic allograft, *de novo* regeneration of cartilage was observed within the graft, as well as renewal of ciliated epithelium in the graft lumen [97,98]. This was followed by a clinical trial with six patients [99]. It was reported that the tracheal replacement with aortic allografts was successful in four of the six patients [99]. In a separate study, five patients who underwent trachea reconstruction with human cryopreserved ( $-80^{\circ}\text{C}$ ) aortic allografts all had favorable outcomes [100]. A similar study reported that thawing cryopreserved aortic allografts enabled viable donor cells to release cytokines and growth factors [101].

Many animal studies have been performed with synthetic 3D-printed scaffolds to determine the materials with the best mechanical properties and also evaluate the effectiveness of graft seeding with autologous cells [102-106]. Kim *et al.* studied the transplantation of a 3D-printed tracheal graft mixed with iPSCs and chondrocytes in a rabbit model [107]. In the study, a tissue-engineered artificial trachea was successfully transplanted into a rabbit model with a 1.5 cm segmental trachea defect. There were no signs of granulation ingrowth in the tracheal lumen, and epithelium and neocartilage successfully formed at the defect sites. According to the research findings by Choi *et al.*, a coating of HA on a hydrophobic tracheal scaffold could improve the adherence of MSCs and tracheal regeneration [108]. In contrast, Pepper *et al.* used synthetic tissue-engineered tracheas in an ovine model. However, it was reported that the transplanted tracheas could not induce optimal epithelialization and neovascularization, and the process led to further complications, such as inflammation and infection [103]. Hence, future studies should focus on scaffold modulation that would accelerate epithelialization and avoid graft devascularization that could lead to graft infection and necrosis.

## 6. Conclusion

Regenerative medicine is rapidly progressing in the field of otorhinolaryngology and has reportedly restored hearing, voice,

and vital functions (e.g., breathing and swallowing) and improved the patient's quality of life. However, the complexity of restoring otorhinolaryngological functions requires further research to refine the techniques used in regenerative medicine.

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## Conflict of Interest

The authors declare no conflicts of interest with regard to the content presented in this work.

## Ethics Approval and Consent to Participate

Not applicable.

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## Availability of Data

Not applicable.

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