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REVIEW ARTICLE

Tissue Engineering and Regenerative Medicine in the Field of Otorhinolaryngology

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Abstract

BACKGROUND: Otorhinolaryngology is a medical specialty that focuses on the clinical study and treatments of diseases within head and neck regions, specifically including the ear, nose, and throat (ENT), but excluding eyes and brain. These anatomical structures play significant roles in a person's daily life, including eating, speaking as well as facial appearance and expression, thus greatly impacting one's overall satisfaction and quality of life. Consequently, injuries to these regions can significantly impact a person's well-being, leading to extensive research in the field of tissue engineering and regenerative medicine over many years.

METHODS: This chapter provides an overview of the anatomical characteristics of otorhinolaryngologic tissues and explores the tissue engineering and regenerative medicine research in otology (ear), rhinology (nose), facial bone, larynx, and trachea.

RESULTS AND CONCLUSION: The integration of tissue engineering and regenerative medicine in otorhinolaryngology holds the promise of broadening the therapeutic choices for a wide range of conditions, ultimately improving quality of a patient's life.

Keywords Otorhinolaryngology · Tissue engineering · Regenerative medicine · Stem cells · Scaffolds · Differentiation

1 Introduction

Otorhinolaryngology (ENT: Ear, Nose, and Throat) is a clinical field that focuses managing diseases occurring in the head and neck region, excluding the eyes and brain. It encompasses various anatomical structures including the nasal cavity, ears, larynx, oral cavity, pharynx, thyroid gland, salivary glands, lymph nodes, and others. Anatomically, the head and neck regions are vital for various special senses such as facial expressions, taste, smell, hearing, as well as everyday activities of eating and speaking. In cases of injuries or anomalies, surgeries could be performed to restore normal structure and function. However, permanent damages or certain anomalies are often challenging to recover from, demanding extensive research in the field of tissue engineering and regenerative medicine (TERM) over many years. Tissue engineering, in conjunction with regenerative medicine has demonstrated a potential in restoring the function of damaged or missing tissues, thereby providing a possible therapeutic option for irreversible diseases.

The term 'tissue engineering' was first introduced in the late 1980s [1], but the concept of creating an artificial organ to replace the function of original tissue started much

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earlier in otorhinolaryngology. In 1949, Parker and his colleagues created an artificial trachea made with vitallium, stainless steel and glass to widen a narrowed airway [2]. Since then, various scaffolds, such as silicone and collagen, have been considered for constructing artificial tracheas and other tissues in clinical medicine [3]. In this chapter, we will review the research on TERM in the field of otorhinolaryngology, focusing on clinical unmet needs and considerations.

2 External ear, auricle

2.1 Clinical considerations

The outer ear, also known as auricle, serves the function of collecting sound and contributes to the overall appearance of the face through its involvement in facial aesthetics. It possesses a relatively simple anatomical structure composed of cartilage, which determines its overall shape, and skin tissue covering it. In adults, the external ear comprises approximately 1–5 million cartilage cells enclosed by a very thin perichondrium [4]. The auricular cartilage is considered one of the most complex three-dimensional (3D) cartilage tissues in the human body among all the cartilage tissues present. The need for external ear reconstruction primarily arises from congenital conditions, such as microtia, in addition to acquired damage due to trauma or tumors (Fig. 1A). Microtia, the most common congenital cause, occurs when the external ear fails to form properly or is partially formed. It is a relatively common facial malformation, affecting approximately one out of every 6,000 newborns [5]. Currently, the standard treatment for microtia involves the use of autologous rib cartilage. This method involves harvesting cartilage from the patient's own rib, shaping it to match the desired form, creating a pocket under the skin in the original ear's location, and transplanting the cartilage [6]. The advantage of using autologous cartilage is the low rate of graft failure, but it requires waiting until a patient to reach the age of 5–6 years, when rib cartilage growth is sufficient, with a risk of complications during the rib cartilage harvesting process.

Efforts have been made over the years to reduce complications associated with rib cartilage harvesting and to explore alternative materials such as silicone, polyethylene, and hydrogel. Silicone, which has been used in clinical practice since the 1950s, was initially popular due to its ease of manipulation and stability over time. However, it poorly integrates with surrounding tissues, and the formation of thick capsules often leads to graft exposure through the skin, decreasing its utilization for ear reconstruction [7]. Subsequently, materials like polytetrafluoroethylene and Gore-tex were employed as implants for ear reconstruction. Gore-tex, with its small pores (10–30 μm), allows better integration with surrounding tissues [8], including blood vessels. However, it loses its shape over time, limiting its use for external ear reconstruction.

In recent years, the most widely used material in clinical practice for ear reconstruction is high-density porous polyethylene (HDPP), marketed under the brand name Medpor® (Fig. 1B). Medpor has a porous structure with pores measuring approximately 100–250 μm, facilitating rapid tissue integration, including blood vessels, from the surrounding tissue [9]. It has a low risk of migration and can be used as early as age 3, unlike rib cartilage transplants. Nevertheless, it is stronger and less elastic than normal ear cartilage, resulting in a different tactile sensation compared to a natural ear. For example, auricular cartilage typically has Young's Elastic Modulus of approximately 1.66 ± 0.63 MPa [7], while Medpor has a Young's Elastic Modulus in the range of approximately 227–307 MPa. This substantial stiffness in Medpor increases the risk of protrusion through the skin in response to external stimuli [7, 10]. To mitigate the risk of graft extrusion, the temporoparietal fascia surrounding tissue must be transplanted along with Medpor during surgery.

2.2 Tissue engineering and regenerative medicine approaches

Unlike most of the cartilage in our body, which is hyaline cartilage, auricular cartilage is classified as elastic cartilage. The auricular cartilage, similar to other elastic cartilages, maintains its shape consistently even under external stimuli in daily life. Therefore, an artificially engineered ear through tissue engineering should possess similar biomechanical properties.

The external ear is one of the earliest artificial organs created in the field of tissue engineering. In 1991, Vacanti et al. first reported their historical tissue engineering approach, the Vacanti mouse [11]. An ear mold was formed in the shape of a 3-year-old child's auricle using a nonwoven mesh of polyglycolic acid after being immersed in a 1% solution of polylactic acid (PLA). Each ear template was seeded with chondrocytes isolated from bovine articular cartilage and implanted into subcutaneous pockets on the dorsa of athymic mice. After 12 weeks of the implantation, the gross morphologic and histologic analyses demonstrated new cartilage formation in the implants. However, the newly formed cartilage was not elastic cartilage, losing its ear shape once the scaffold materials naturally degraded.

Achieving a balance between maintaining shape and strength while allowing appropriate elasticity is a challenge in the development of artificial ears, since it requires

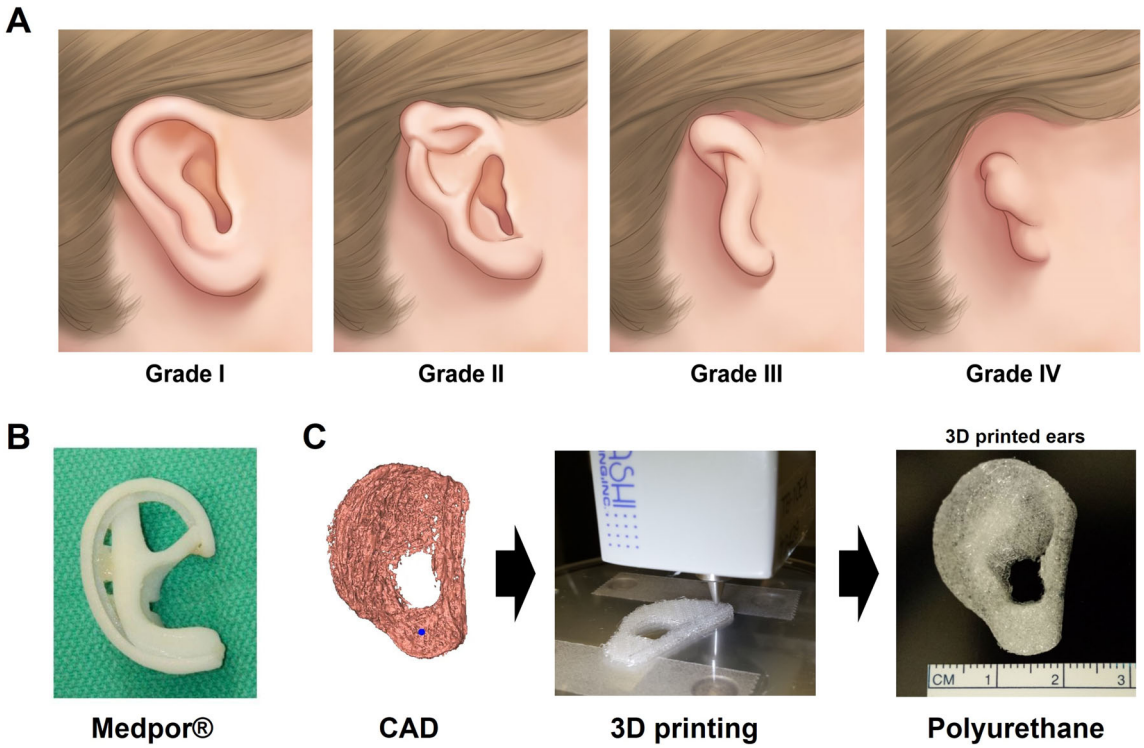

Fig. 1 **A** Microtia–congenital ear deformation at different stage. The ear fails to develop fully during first trimester. Grade 1. The ear looks smaller but still resembles the physiological structure of a normal ear. Grade 2. The ear still maintains normal features but missing some features, such as ear canal and some portion of upper ears. Grade 3. The ear lobe is present at different position. Only small remnant of cartilage and soft tissues remains, resulting in aural atresia. Grade 4. Also known as anotia, which is characterized by total absence of the ear. **B** Medpor® is made with high-density porous polyethylene (HDPP) and most commonly used for ear reconstruction in clinical practice. **C** A normal ear is scanned using a computed tomography or magnetic resonance imaging system, followed by computer-aided design (CAD) to recreate the shape of the ear. 3D printing is then used to construct a personalized artificial ear with various scaffolds such as polyurethane

mimicking of the unique biomechanical properties of elastic cartilage found in the human ear. In the past, a hydrogel- or polymer-based support structures with cartilage cells were injected into the molds of the ears to create an ear reconstruct [12]. However, these methods encountered difficulties in achieving complex ear shapes that perfectly match the symmetry of the opposite ear, often requiring separate molds for each case. Therefore, significant research has been conducted using computer-aided design and computer-aided manufacturing (CAD/CAM) alongside bio-fabrication techniques [13] (Fig. 1B). The process typically involves scanning the shape of a normal ear using computed tomography (CT) or magnetic resonance imaging (MRI) [14]. Subsequently, the scanned image is mirrored to recreate the shape of the ear on the side requiring reconstruction [15]. Finally, 3D printing is used to create an artificial ear that matches the desired shape [4, 16] (Fig. 1B). This approach offers several advantages over previous methods, as it allows for the precise and uniform fabrication of support structures, or scaffolds, and enables the utilization of various types of biomaterials and stem cells for constructing the ear [17–19]. There are still numerous challenges with 3D-printed artificial ears with safety as well as legal and ethical issues of biomaterials and human stem cells [20].

3 Inner ear, cochlea

3.1 Clinical considerations

The inner ear, located within the temporal bone, consists of the cochlea responsible for hearing and three semicircular canals and the vestibule responsible for balance (Fig. 2). The cochlea, also known as the snail-shaped organ, earns its name from its resemblance to a snail shell. In humans, the internal structure of the cochlea can be divided into three parts: Reissner's membrane, the basilar membrane, and the osseous spiral lamina. Reissner's membrane and the basilar membrane together form a central component known as the cochlear duct or scala media [21]. Inside this duct, the Organ of Corti, responsible for hearing, is located. The Organ of Corti includes hair cells and supporting cells. The supporting cells provide structural support for the hair

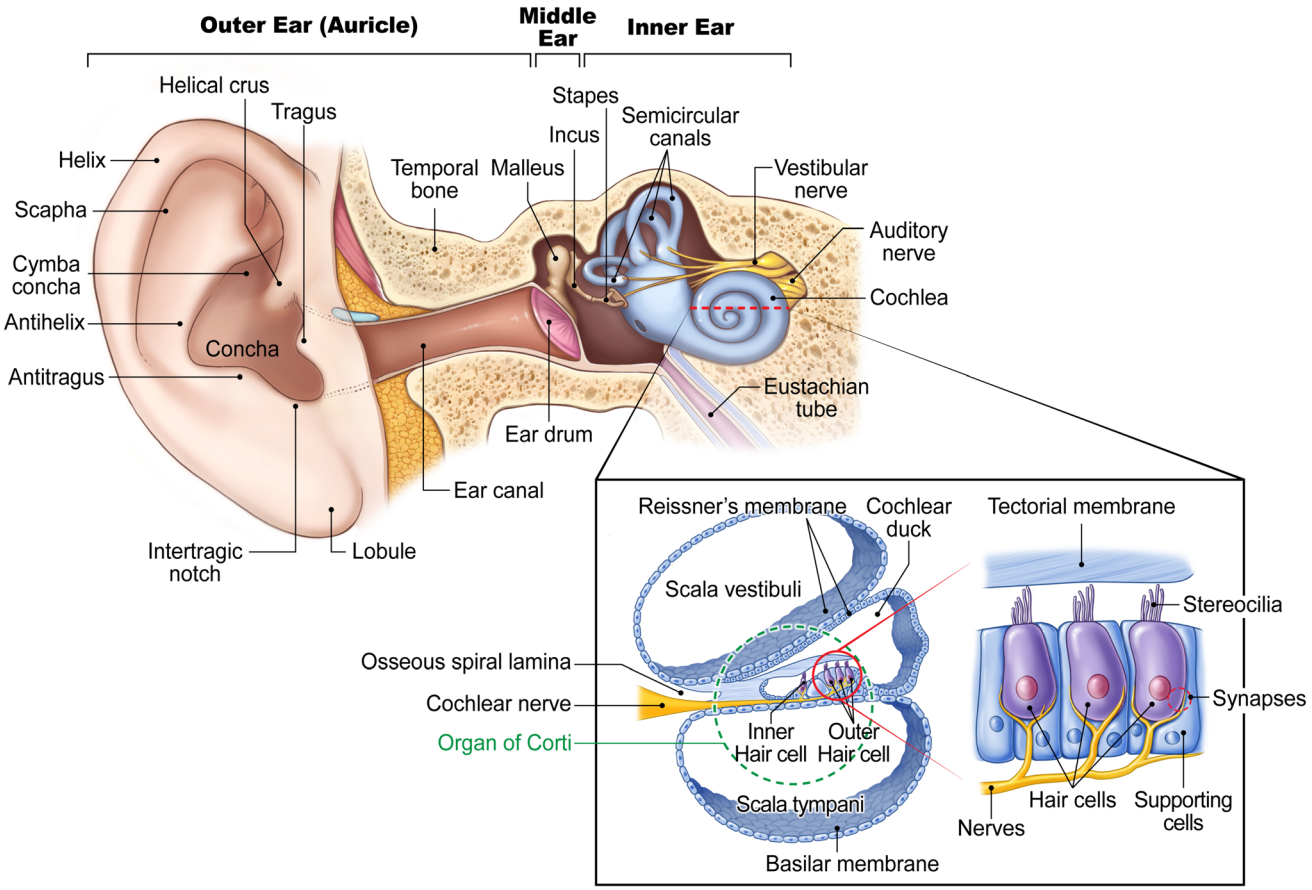

Fig. 2 The structure of auditory system. The ear has the outer, middle, and inner ears. The outer ear (auricle) collects and amplifies vibrations (sound wave) and channels into the ear canal. The middle ear transmits the acoustic vibrations from tympanic membrane to cochlear. The inner ear transforms vibration into electrical signals through nerve impulses. Cochlea is responsible for hearing and three semicircular and vestibule are essential for balance. The cochlea has duck known as the Organ of Corti, which contains sensory cells containing hair cells for auditory function. Auditory neurogenic stem cells can be found in this area, but their availability is limited

cells, and the hair cells converts vibration into electrochemical nerve impulses through stereocilia. Inner hair cells are the primary sensory organ that transfers information of received sound wave to the central nervous system, while outer cells receive inputs from the brain, modulating the function, adjusting tuning and intensity of sound perceived by inner hair cells, through manipulation of the resonance of perilymph fluid movement in the scala media [22]. These hair cells can be easily damaged by aging, loud noise, medicine, infections, and many other idiopathic illnesses.

The prevalence of hearing impairment is relatively high with approximately 10–14% of the global population experiencing some form of hearing impairment at some point in their lifetime [23], though the severity may vary. In general, spontaneous improvement in hearing is assessed for the first few weeks after onset of symptom using audiogram. If hearing does not recover on its own, intratympanic steroids are directly injected into middle ear to decrease inflammation and/or oedema in the hearing organs, especially in the cases of idiopathic sudden sensorineural hearing loss [24]. Hearing aids are the most common treatment used in chronic cases, and cochlear implants have also been used to bypass damaged hair cells [25]. However, in cases of severe damage of auditory neurons, drug delivery of neurotrophins in conjunction with Gelfoam™ or Microwick™ to cochlea are being employed to promote survival and to regenerate damaged hair cells [26]. Unfortunately, all mammals, including humans, lack the regenerative capacity to fully restore the function of damaged sensory cells in the cochlea once they fully developed. While there are limited numbers of progenitor or stem cells believed to exist within the cochlea [27], their differentiation and regenerative abilities are known to be less robust compared to the stem cell derived from other tissue sites. This limited regenerative capacity within the cochlea challenges in restoring hearing function once it has been compromised.

3.2 Tissue engineering and regenerative medicine approaches

Research in regenerative medicine for hearing loss primarily focuses on cell-based therapies using stem cells. While damage to hair cells in the Organ of Corti is generally considered irreversible, some studies have observed evidence for the regeneration of hair cells in the vestibular system, suggesting the presence of stem cells within the auditory organ [27, 28] (Fig. 2). In fact, successful isolation of stem cells from various parts of the cochlea, vestibular system, eardrum, and the cochlear nerve ganglion has been achieved in many animal models [27, 29–31]. However, obtaining autologous stem cells from the inner ear is challenging due to their limited availability and accessibility. As a result, most stem cell-based therapies for restoring hearing function are based on allogeneic embryonic stem cells or adult stem cells.

Experiments involving the transplantation of adult neural stem cells isolated from the hippocampus or periventricular regions of the central nervous system into animal models of hearing loss have demonstrated their ability to differentiate into specific cell types that support the function of the sensory epithelium and cochlear nerve ganglion [32]. Similarly, bone marrow-derived mesenchymal stem cells (BM-MSCs) have shown the capacity to differentiate into the cells that retain the characteristics of specific neuronal and neural crest markers under a suitable microenvironment [33]. BM-MSCs have also shown to improve the hearing threshold in patients with ototoxic sensorineural hearing loss [34, 35]. Additionally, alongside the therapies involving differentiated cells, research also suggests the paracrine effect of neural stem cells, which can mitigate ischemic damage to the cochlea, reducing the hair cell damage [36, 37].

Indeed, the inner ear presents unique challenges for research due to its small size, making it difficult to develop an animal model. Moreover, direct transplantation of stem cells into the inner ear is a challenging task both in animals and humans. Additionally, the high potassium concentration in the endolymph of the inner ear poses a challenging environment for the survival of transplanted cells, necessitating careful consideration in such therapies.

4 External nose and paranasal sinuses

4.1 Clinical considerations

The nose (external nose) and paranasal sinuses serve as the first gateway for the respiratory system, through which approximately 10,000–20,000 L of air pass daily [38]. The surfaces of the nasal cavity and paranasal sinuses are covered with pseudostratified ciliated columnar epithelium [39]. The mucous glands produce around 100–200 mL of mucus daily to maintain the appropriate humidity of incoming air. This microenvironment also allows the smooth movement of cilia, which prevents the entry of small foreign particles into the respiratory system. More than 90% of these particles are expelled to the outside through coughing facilitated by mucus and ciliary action or they are swallowed and transported to the digestive system.

The nasal cavity is divided into two sides by the nasal septum. Each side of nasal cavity can be further sectioned into four paranasal sinuses: the frontal sinus, ethmoid sinus, sphenoid sinus, and maxillary sinus. These sinuses secrete mucus to protect against infection and to main humid environment for humidifying and heating inhaled air before it reaches the lung. Within the upper mucosa of the nasal cavity lies the olfactory system, plays a role in smelling and detecting odors (Fig. 3). The olfactory system includes olfactory filament, bulb, and tract. The olfactory filament consists of a cluster of thousands of olfactory receptor axons extended from primary olfactory sensory neuron located within olfactory epithelium. These neurons form olfactory nerves, also known as cranial nerve II (CNII), which connects to the central nervous system through olfactory bulb and tract.

Clinical approaches largely vary depending on prognosis. For damaged nasal cavity, nasoseptal reconstruction and/or septoplasty is performed to optimize airflow. However, there is currently no established treatment for olfactory loss from paranasal sinus issues. While corticosteroids have been used to temporarily improve olfactory function [40], maintaining intranasal steroids has been shown to improve olfactory function, with few report of side effects, including headache, nasal infection, and epistaxis [41, 42].

4.2 Tissue engineering and regenerative medicine approaches

The nasal septum, with the exception of the L-strut (Fig. 3), provides a significant amount of cartilaginous tissue often utilized for reconstructive surgeries requiring cartilage, such as auricular and rib cartilage [43]. A septoplasty and/or nasoseptal reconstruction are common practices to correct optimal function of the nose such as breathing. A septoplasty manipulates existing bone and cartilage to improve the airway, while nasoseptal reconstruction often involves with extensive repair of the airway, requiring grafting and reshaping of cartilage. In situations where there is not sufficient cartilage available for reconstruction, cartilages from other body parts, such as the auricular cartilage, or PCL-based nasal meshes have been clinically used. Other artificial scaffolds, including

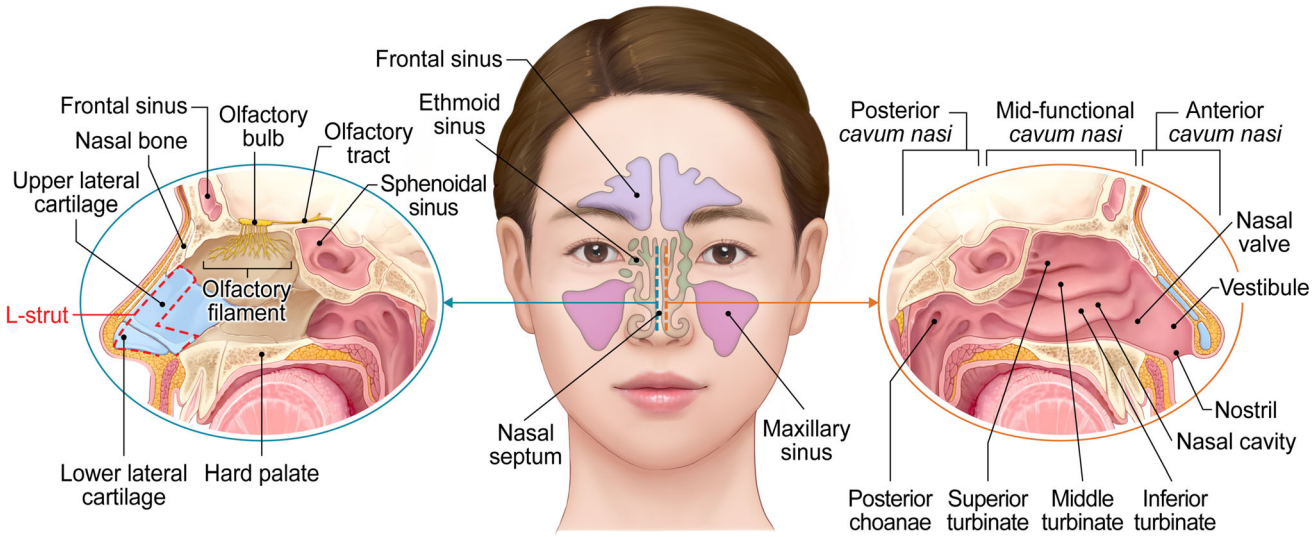

Fig. 3 The structure of the olfactory system. The nose generally has four pairs of sinus cavities, including frontal sinuses located in forehead, sphenoid and ethmoid sinuses between the eyes and nose, and two maxillary sinuses behind the cheekbones. The nasal septum in the midline of the nose contains a hyaline cartilaginous tissue that provides structure support of the nasal cavity and separates the right and left nostrils. It is often used for a cartilage reconstructive surgery. Turbinates (superior, middle, and inferior) are bone structure that regulate airflow and humidity in the nasal cavity. The olfactory nerves are located at the upper mucosa and the nasal cavity to detect smell and provides stem cells with neurogenic potential

collagen [44, 45], hydrogels [46], PLGA [47], and polyurethane [48], have also been tested as possible replaceable materials. To match the desired shape and size, ensuring precise customization for each patient, CAD/CAM are utilized to fabricate personalized 3D scaffolds [49].

The olfactory mucosa, which houses the olfactory nerve, contains specific stem cells related to nerve differentiation in its vicinity [50, 51] (Fig. 3). These olfactory stem cells are named as 'olfactory ecto-mesenchymal stem cells (OE-MSCs)' due to their high osteogenic and neurogenic potentials [52]. OE-MSCs can be activated in response to damaged olfactory neuron, restoring the sense of smell [53], and therefore, transplantation of OE-MSCs or olfactory epithelial cells/mucosa containing these cells has been suggested as a possible therapeutic option for restoring olfactory function. In fact, Kurtenbach et al. (2019) reported that the engraftment of OE-MSCs restored electrophysiologic odor responses and olfactory behavior level in hyposmia mice model [54]. Due to the high neurogenic potentials of OE-MSCs, several studies have used OE-MSCs as a possible cell source for regenerating damaged hippocampal neurons of neurodegenerative diseases [52, 55]. However, the quantity of obtainable OE-MSCs is limited in humans, thereby limiting its clinical application. Therefore, some studies have reported of using MSCs derived from bone marrow [56, 57] or adipose tissues [58] to restore olfactory function (Table 1).

5 Facial bone

5.1 Clinical considerations

The craniofacial and maxillofacial regions are areas of medical practice that extend beyond otolaryngology. They are also managed by other clinical specialties, including neurosurgery, plastic surgery, as well as oral and maxillofacial surgery. For instance, in cases where malignant tumors originate in the frontal sinuses and invade the skull base above or extend to the skin of the facial area, complete excision of the tumor may require the removal not only of the facial skin but also parts of the sinuses, nasal passages, and even sections of the brain. Therefore, the treatment of such tumors involves multiple specialties working together from the outset, and the reconstruction phase must also meet the specific clinical requirements of each specialty.

Reconstruction of the facial structure often involves sourcing tissues from other parts of the body that have histologically identical tissues, such as bones, cartilage, muscles, and nerves. For example, in case requiring surgical excision of a small cancerous legion (generally less than 2–3 cm) of mandible (lower jar), bone grafting can be performed without vascular anastomosis, with success rates ranging from about 70–88%. However, when the extent of bone damage exceeds 5–6 cm in length, the patient has received radiation therapy, or the central part of the mandible is involved, the success rate drops dramatically [59]. In such cases with extensive damage, vascularized

Table 1 MSCs used for restoring sensory functions within the ear and nose

Types of MSC	Animal model	Cell type to transplant	Functions	Refs
Human BM-MSCs	*In vivo* acute ototoxic deaf animal model	MSCs	Functional recovery of ototoxic hearing loss	[34]
Mouse BM-MSCs	*In vivo* spinal ligaments degeneration C57BL/6 J mice model	MSCs	1) Accelerated regeneration or maintenance of fibrocytes in damaged spinal ligaments 2) Partial functional restoration of the mouse cochleae	[35]
Human BM-MSCs	*In vivo* the cochlea of an auditory-neuropathy guinea pig model	Neural-induced MSCs	1) Restoration of damaged spiral ganglion neurons 2) Decrease hearing thresholds in an auditory-neuropathy model	[37]
Mouse OE-MSCs	*In vivo* hyposmia mice model	MSCs	Restoration electrophysiologic odor responses and olfactory behavior level in hyposmia mice model	[54]
Mouse BM-MSCs	*In vivo* Balb/C AJc1-nu/nu mice model	MSCs	Inducing differentiation of transplanted cells into premature olfactory receptor neurons	[56]
Mouse BM-MSCs	*In vivo* Purkinje cell degeneration mutant mice model	MSCs	Generation of large numbers of microglial cells in the olfactory bulb and reduction of degenerative processes	[57]
Rat A-MSCs	*In vivo* traumatic anosmia rat model	MSCs	1) Inducing differentiation of transplanted cells into olfactory receptor neurons and endothelial cells 2) Regeneration of olfactory epithelium	[58]

BM-MSCs, bone marrow-derived mesenchymal stem cells; OE-MSCs, olfactory ecto-mesenchymal stem cells; A-MSCs, adipose-derived mesenchymal stem cells

grafts, such as fibula free flaps, are used for reconstruction. The success rate of fibula free flaps is very high, having approximately 91–99% of success rate. The fibula free flap includes not only a sufficient skin graft but also a segment of the fibular bone, approximately 25 cm in length, which allows for future implantation of artificial teeth [60, 61].

However, despite its high success rate, factors such as complications from fibular bone harvesting, prolonged surgery times, extended hospital stays, and the difficulty of accurately replicating facial aesthetics remain challenges [60, 62]. In this context, regenerative medicine approaches offer ideal solutions. Rapid prototyping models have been widely used in clinical practice for facial bone reconstruction. Based on CT images taken before surgery, the location and extent of tumor excision can be predetermined. A model or prototype is created before surgery to assist with flap reconstruction, ultimately saving surgical time.

5.2 Tissue engineering and regenerative medicine approaches

Regenerative medicine research in craniofacial bones, like other bones in the body, must be tailored to the specific anatomical and functional characteristics of each area. For example, long bones, such as those in the legs, must possess sufficient strength to bear the weight of the body (weight-bearing bones) [63]. In contrast, cranial bones, such as the frontal and parietal bones, primarily serve a cosmetic role and do not require the same level of strength. The ethmoid bone, which forms the inner aspect of the orbital cavity, is extremely thin, and in some cases, it may not require reconstruction even if it is removed during sinus surgery. On the other hand, the mandible, being a load-bearing bone, must withstand the pressures generated during activities like chewing and also allow for the placement of dental prostheses. Therefore, research into regenerating facial bones should consider the unique requirements of each anatomical region (Fig. 4).

Research in facial bone regeneration, similar to other bone regeneration studies, involves not only the use of ceramics or bone-mimicking materials but also the combination of various elements such as biomaterials, growth factors, stem cells, and scaffolds to achieve optimal biomechanical properties [64]. Common biomaterials used include ceramics [65], polycarprolactone (PCL), PLA [66], gelatin [67], hydroxyapatite [68], hydroxyapatite/beta-tricalcium phosphate [69, 70], polymethylmethacrylate [71], all of which closely mimic bone structure. Growth factors like bone morphogenetic protein (BMP), which has demonstrated effectiveness in nonunion fracture treatment [72], are commonly used [73, 74]. Various stem cells are

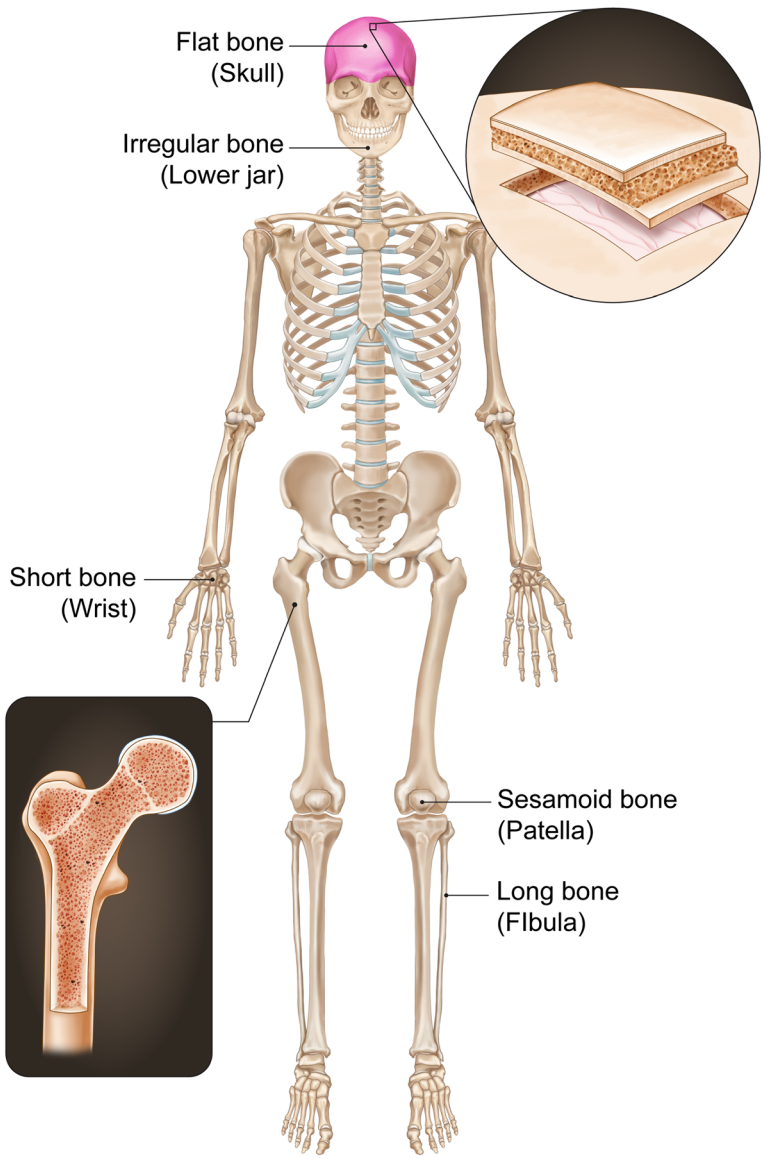

Fig. 4 There are five general types of bone in the human skeletal system: flat, long, short, irregular, and sesamoid bones. The flat bones are located in the skull, thoracic cage, and the pelvis. They are composed of two thin layers of compact bone covering the spongy bone (marrow) in the middle to protect the internal organs such as the brain, heart, and pelvic organs. The long and short bones are the compact bones surrounding the spongy marrow region in the center. The long bones are located in the lower and upper limbs of appendicular skeleton and serve the function of supporting the weight of the body and facilitating movement. The short bones are cube-shaped bones that are located in the wrist and ankle joints to maintain the stability and movements of the body. The irregular bones have various complex shape and structure that have a function of protecting the internal organs, such as spinal cord and vertebral column. The sesamoid bones are small and round bones that are embedded in the tendons to protect and to reinforce the tendons

employed as cellular sources; while early research primarily used BM-MSCs [75], recent studies have focused on stem cells which are readily available in large quantities, such as adipose stem cells [76, 77] and tonsil-derived mesenchymal stem cells.

In 2006, Warnke et al. reported a successful case, where BM-MSCs combined with BMP-7 were applied to a scaffold composed of titanium mesh and hydroxyapatite and transplanted into the latissimus dorsi muscle [75]. This construct induced bone formation and was successfully transplanted into a patient with mandibular bone damage caused by malignant tumors. Regenerating flat cranial bones, such as the maxilla, is somewhat simpler than reconstructing weight-bearing bones like the fibula. In these cases, using a porous titanium mesh or polymethylmethacrylate as a scaffold, along with the natural ingrowth of cells, blood vessels, and fibrous tissue from the surrounding healthy tissues, is often sufficient for effective reconstruction [78]. Since the shape of facial bone varies greatly depending on each individuals, CAD and 3D-printing have been actively utilized to create optimized shapes for individuals [66, 79].

6 Larynx

6.1 Clinical considerations

The larynx is responsible for respiration, phonation, and swallowing. It consists of vocal folds, along with the thyroid and cricoid cartilages, which enable active vocal fold movement. The lining of the larynx is covered by

respiratory epithelium, which covers part of the airway, while the vocal folds are covered by squamous epithelium. Beneath the squamous epithelium, the vocal folds consist of three layers of the lamina propria (superficial, middle, and deep layers) and the thyroarytenoid muscle [80]. The lamina propria plays a cushion-like role, ensuring smooth vibration of the vocal fold mucosa. During respiration, the vocal folds open outward to facilitate the flow of air. During phonation, they come together medially, causing the vocal fold mucosa to vibrate, producing sound. Moreover, during swallowing, the epiglottis, false vocal folds, and true vocal folds close, and the entire larynx rises, preventing food from entering the airway.

6.2 Tissue engineering and regenerative medicine approaches

There are two significant laryngeal disorders currently being treated through a regenerative medicine approach: unilateral vocal fold paralysis and vocal fold scar. Unilateral vocal fold paralysis results from the immobilization of one vocal fold, leading to a breathy voice during phonation and the risk of aspiration during eating. It is typically caused by damage to the recurrent laryngeal nerve, which controls vocal fold movement, and can also be associated with fixation of the cricoarytenoid joint. In cases of unilateral vocal fold, laryngeal framework surgery in conjunction with the injection or implantation of various autologous or artificial materials has been suggested as the standard treatment for recovering normal vocal fold vibration [81].

When the movement of vocal fold is paralyzed, a vocal fold medialization procedure such as type I thyroplasty can be performed to reposition the paralyzed vocal fold to the midline position [82]. Injection laryngoplasty involves injecting a substance into the lateral side of the vocal folds from the outside to medially reposition them (Fig. 5). Back in 1911, paraffin was initially used as the injecting material for larygoplasty injection [83]. Since then, other injectable materials, including Teflon [84], calcium hydroxylapatite [85], autologous fat [86], Artecoll® [87], silicone [88], and collagens [89], have also been used to increase the efficacy and safety of the procedure. Each injection material has its own advantages and disadvantages (Fig. 6).

Vocal fold scar, generally caused by inflammation, trauma, or phonosurgery, is one of the most challenging conditions to manage in laryngology. It involves damage to the layered structure of the vocal fold mucosa, impairing the formation of a normal vibratory waveform and causing voice-related issues [90]. When scar tissue forms between the vocal fold mucosa and the thyroarytenoid muscle, the layer structure disoriented, and the vocal fold mucosa cannot vibrate properly, leading to voice abnormalities. Vocal fold scarring increases the deposition of type 1 collagen in the vocal fold lamina propria and decreases hyaluronic acid, elastin, decorin, and fibromodulin [91]. Regenerative medicine approaches to treat vocal fold scarring are primarily focused on reducing fibrosis and scar formation due to collagen deposition and restoring the extracellular matrix (ECM) composition in the lamina propria [92]. Growth factors such as basic fibroblast growth factor (bFGF) [93], hepatocyte growth factor (HGF) [94], and transforming growth factor (TFG) [95] have been reported to decrease vocal fold scarring and assist in restoring ECM composition. Mesenchymal stem cells derived from adipose tissues were also shown to be effective in reducing vocal fold scarring [96].

7 Trachea

7.1 Clinical considerations

The trachea is an anatomical structure that extends from the larynx to the lungs, connecting the upper and the lower respiratory tract. In adult, the trachea is approximately 15–17 cm long and has a cylindrical shape composed of 15–20 C-shaped cartilaginous rings that provide structural support. The tracheal rings are made of hyaline cartilage and are connected by muscles (trachealis) between each ring. On the posterior side of each ring, there is fibrous tissue (annular ligament of the trachea) that runs vertically, providing flexibility and elasticity for body movement or the cough reflex. The inner lining of the trachea is covered by respiratory mucous epithelium, which contains cilia that assist in moving mucus to trap and expel bacteria or foreign particles entering from the external environment.

Tracheal defects can be classified as congenital or acquired. Congenital conditions generally occur due to tracheal malformations or congenital stenosis, while acquired injuries are typically due to trauma, tumor invasion, or inflammatory conditions, causing stenosis. They can also be classified according to the extension and size of partial or circumferential defects. In cases of minor injuries, such as tearing, a simple primary closure can be carried out (Fig. 7). A long circumferential tracheal injury or stenosis can be treated with surgical procedures, such as end-to-end anastomosis [97]. In end-to-end anastomosis, the damaged portion of the trachea is removed, and the healthy tissues are rejoined together. This procedure could be used for tracheal injuries with less than about 6 cm in length (50% of the length of adult trachea), yet this would require additional procedures to reduce tension between the connected tracheal rings, which may require patients refraining their neck for about two weeks.

Procedures	Injection laryngoplasty	Type I thyroplasty
Indications	Temporary correction of glottic incompetence due to unlateral vocal fold paralysis and long-term correction of mild-moderate glottic insufficiency	Permanent correction should be reserved for cases of vocal fold paralysis in which recovery of motion is definitively not expected (time>6months from onset, surgical recurrent nerve sacrifice, or malignanat invasion)
Techniques	Percutaneous, transnasal or peroral injection of filler material into the vocal cord or paraglottic space	Insertion of an implant into the vocal fold through a window in the thyroid, which results in displacement of the paralyzed vocal fold to a more medial position

Figure

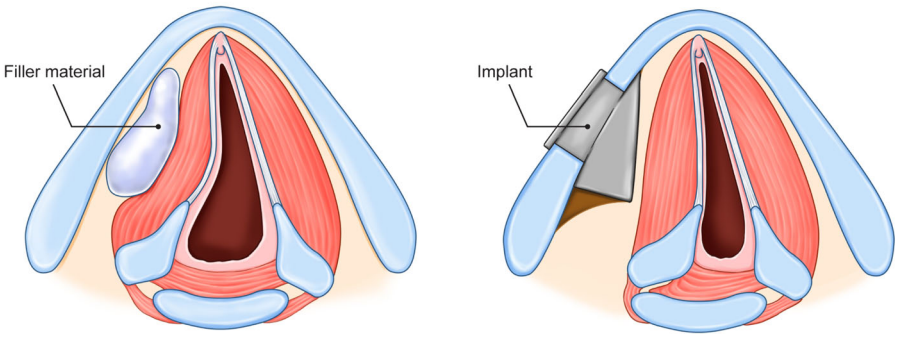

Fig. 5 Medialization procedures used for correcting vocal fold paralysis. Injection larygoplasty involves injecting various substances (paraffin, collagens, silicones…etc.) into the lateral side of vocal fold or paraglottic space to medially repositing the fold, providing immediate but temporary correction. Type I thyroplasty is the most common surgical procedure for achieving permanent correction of vocal fold paralysis. It involves inserting implants (silicones, titanium, Teflon…etc.) into the vocal fold to restore symmetry and functionality

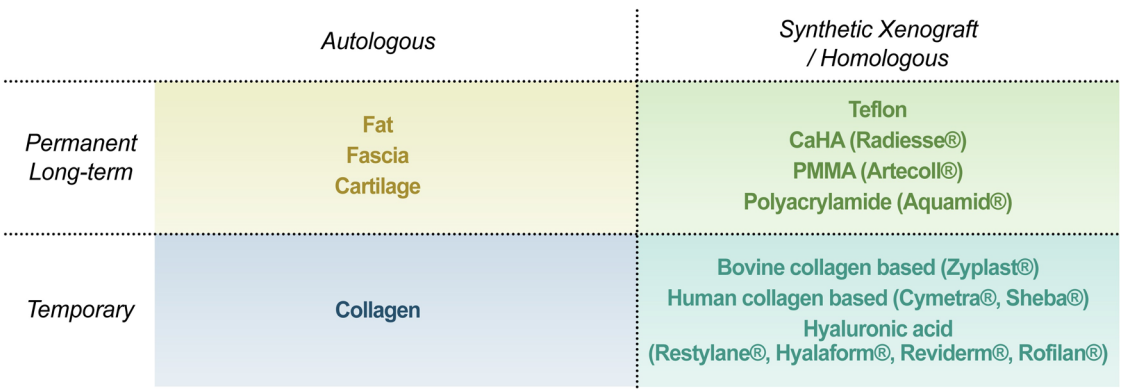

Fig. 6 Various injecting materials used in larygoplasty to correct the vocal fold paralysis include autologous materials, such as fat, fascia, cartilage, and collagen, which offer advantages in biocompatibility. Synthetic materials, such as Teflon, calcium hydroxylapatite (CaHA), polyacrylamide, hyaluronic acid. offer higher durability and ready availability, despite having their own potential drawbacks. The choice of injecting materials is largely dependent on patient preference, surgeon expertise, and the conditions of the vocal fold paralysis

Clinical challenges are often encountered in treating injuries with intermediate-sized partial defect, typically in the range of 1 cm^2, which is the most common situation in clinical practice (Fig. 7). An end-to-end anastomosis is too extensive procedure for managing a lesion of this size. Primary closure is not applicable due to the elasticity of the

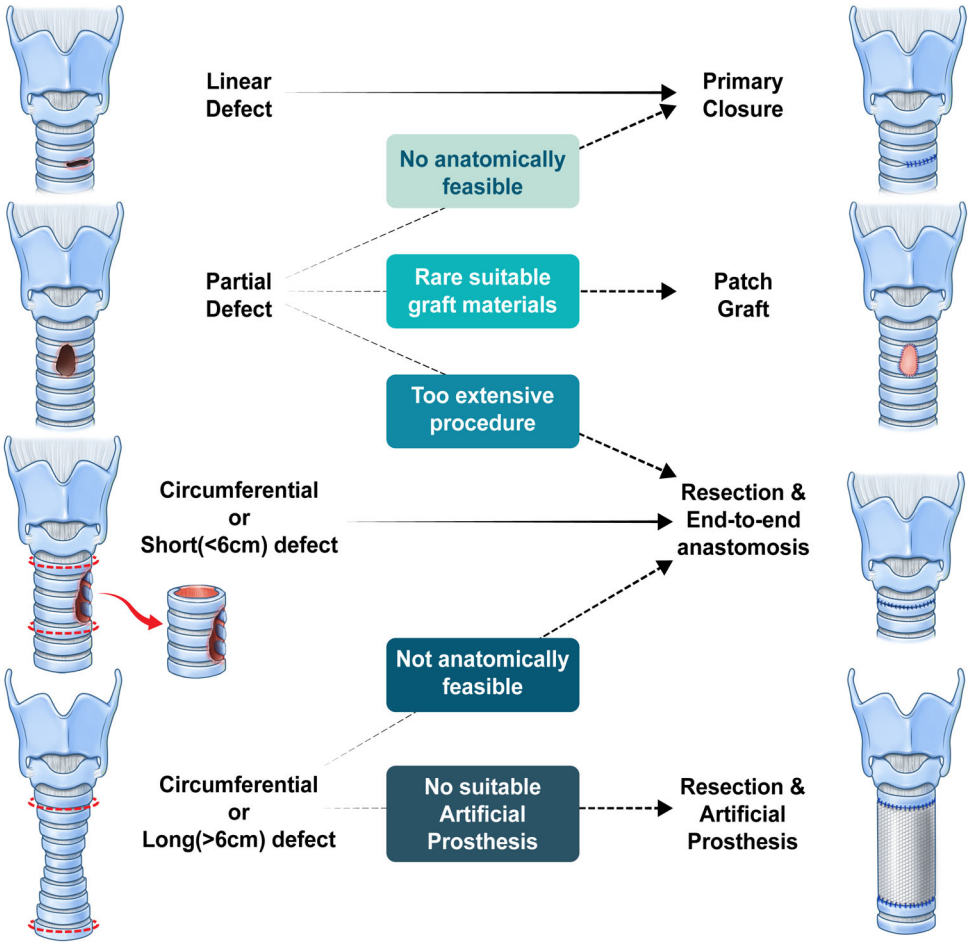

Fig. 7 The treatment approach varies depending on the type of tracheal defect. For minor linear tearing, a primary closure is sufficient. However, dealing with partial defects, typically in the range of 1 cm^2 may pose challenges. When a primary closure is not feasible, patch grafting with muscle or cartilage is a common alternative treatment. Extensive injuries spanning less than 6 cm in circumference require tracheal resection and end-to-end anastomosis. However, defects exceeding 6 cm in length mandate the use of an artificial prosthesis

trachea. In such cases, patch grafting using muscle, fascia, or cartilage, is commonly used [98]. Nevertheless, these grafts are susceptible to infection and may not withstand respiratory pressures, leading to a higher risk of treatment failure. In the case of a circumferential injury to the trachea that exceeds 6 cm in length, tracheal resection and end-to-end anastomosis are not anatomically feasible (Fig. 7). For these reasons, the development of tissue engineering therapies should focus on the partial and long-length reconstruction of the trachea using artificial prostheses.

7.2 Tissue engineering and regenerative medicine approaches

The trachea, despite its seemingly simple cylindrical shape, presents significant challenges for tissue engineering and regeneration due to its unique anatomical and physiological characteristics [99]. One of the major challenges in tracheal regeneration is the need to recreate the complex structure of the trachea, which consists of an outer cartilaginous supporting framework and an inner functional-respiratory mucosa barrier. The tracheal cartilage forms a hollow cylindrical structure designed to uphold the integrity of trachea under fluctuating respiratory pressure and to provide a surface for the attachment of mucous epithelium. Moreover, the trachea has the unique properties and histological characteristics, including respiratory mucous epithelium and cartilage, requiring maintenance of flexibility, optimal elasticity, and strength to withstand the change in air pressure [100, 101]. Additionally, the trachea is exposed to the external environment through respiration, making it susceptible to continuous exposure to external air and foreign particles, all of which should be considered in the tracheal reconstruction process.

Various materials have been employed as scaffolds to maintain the structural support of the trachea. These materials include: polyglycolic acid (PGA), poly-lactic-co-glycolic acid (PLGA), polyester urethane, polyethylene oxide-terephthalate/poly-butylene terephthalate (PEOT/PBT), gelatin sponge, polytetrafluoroethylene (PTFE), as well as biocompatible materials like collagen, chitosan, hyaluronic acid and polyurethane [98, 102–104]. Limited

Table 2 Application of 3D scaffold in otorhinolaryngology

Materials	Printing platforms	Target region	Advantages	Disadvantages	Refs
PU	FDM	Auricle	Highly elastic property, flexibility, microporous structure	Long-term behavior of implanted	[16]
Titanium (Ti64) and polyamide (PA12)	DMLS	Nasal septum	Safe and stable coverage, high resistance to shrinkage forces	Potential risk of exposure	[49]
PLA	FDM	Calvarial bone	Biodegradable with superior chemical, physical, and mechanical properties; enhanced strength	Short degradation rate	[67]
PLA	FDM	Mandibular bone	Clinical-grade, easy control of architecture	Weak osteoinductive effect	[66]
PCL	SLS	Mandibular bone	Biological similarity to the mandible, bioresorbable	Lack of bone formation in the center of the scaffold, long degradation time (greater than 2 years)	[69]
PLCL	pMSTL and IMS	Trachea	Feasibility, appropriate mechanical behavior	Non-clinical use	[106]

PU, polyurethane; PLA, poly-lactic acid; PCL, polycaprolactone; PLCL, Poly(L-lactide-co-ε-caprolactone); FDM, fused deposition modeling; DMLS, direct metal laser sintering; SLS, selective laser sintering; pMSTL, projection based micro-stereolithography

blood supply leads to insufficient regenerative capacity for cartilage and epithelium. Therefore, the scaffold material should possess biocompatible properties, especially conductive to angiogenesis. Some scaffolds have porous structure that allow the formation of blood vessels and attachment of mucous tissues [105], yet it would be difficult for these porous scaffolds to have airtightness that withstand air pressure during airflow. Therefore, researchers have focused on designing scaffolds with biocompatible porous structure. In recent development, 3D printing techniques and the use of decellularized extracellular matrix (ECM) obtained from cadaveric trachea have emerged as promising method for constructing bio-compatible materials to improve the integration of the implant in the host [106].

Differentiating epithelial cells into respiratory epithelium, including mucous and ciliated cells, are another requirement for regenerating trachea tissues. Successful cultivation of respiratory epithelium requires several conditions, including the formation of a basal lamina with a collagen fiber base, growth factors secreted by fibroblasts, and a culture environment resembling the actual air-fluid environment of the airway [107, 108].

8 Conclusion

It has been almost 40 years since the first introduction of tissue engineering, tissue engineering in otorhinolaryngology has been utilized for a much longer time [1, 2]. Tissue engineering and regenerative medicine in otorhinolaryngology have opened up wide treatment options for patients with significant ENT injuries and congenital anomalies. Scaffolds made from materials such as hydrogels, collagens, silicones, and various polymers have been developed to mimic the structural support provided by bones and cartilage, especially for tissues in the external ear, nose, facial bones, trachea, and larynx. These scaffolds are used in conjunction with cells, typically stem cells, as well as cellular byproducts, such as exosomes, to restore and replace damaged or impaired tissues in the hair cells of the inner ear (for hearing), olfactory nerves from the paranasal sinuses (for smelling), and epithelium of the larynx and trachea (for speaking). Nonetheless, many challenges remain due to the difficulty in precisely replicating functionalities owing to the complexity of organs and tissues [20].

For translating innovative approaches from basic research to clinical application in tissue engineering, numerous factors such as functionality, scalability, regulatory hurdles, and safety, should be carefully considered [109, 110]. Functionality and scalability prioritize the effectiveness of products, while regulations emphasize safety as their primary concern. In general, a biomaterial must possess biodegradability with minimal biological activity to meet standard requirements [20]. If a material has additional functionalities, then different requirements are applied. In tissue engineering, across various medical fields including ENT, there has been a growing interest in incorporating stem cells into scaffolds to enhance

regeneration capacity [64]. However, this could complicate the requirements in FDA approval processes. To minimize the complexity of regulatory hurdle, a scaffold with already-approved materials like collagen are often being used, yet conventional polymers often fail to replicate biological niches of original tissues, implicating the need for developing more compatible polymers. Our review has highlighted some advanced biomaterials with promising phenotypical characteristics of newly designed biomaterials, such as polyurethane and polymethylmethacrylate, for the reconstruction of the ear [104], nose [48], and facial bones [71]. Nonetheless, they encounter challenges in meeting FDA safety standards and regulatory requirements due to complexities of FDA regulations. Therefore, a clear guidance from FDA authorities regarding the clinical application of these innovative biomaterials is essential to further advance tissue engineering.

Another avenue for advancing tissue engineering in otorhinolaryngology lies in addressing gaps in current knowledge. This involves comprehension of complex interactions between biomaterials and host tissues in the unique anatomical structures and their corresponding functions of the ENT. Researchers are increasingly exploring materials that closely mimic microenvironments of native tissue, aiming to improve cell adhesion, proliferation, and differentiation [52, 87, 111]. Bioactive polymers, nanomaterials, and/or decellularized scaffolds have been designed to enhance structural and functional outcomes for tissue regeneration. Emerging technologies such as artificial intelligence (AI), 3D-pritning, and CAD, also offer precise control of scaffold architecture, porosity, and mechanical properties, allowing personalized constructs that meet the needs of individual patients (Table 2). The integration of patient-specific imaging data into CAD, assisted by artificial intelligence (AI), facilitates the development of personalized implants, optimizing both fit and function for enhancing tissue engineering in the field of otorhinolaryngology [13, 49] (Fig. 1B). This would eventually expand the range of treatment options available to patients, potentially leading to substantial recovery or even further improving the natural biological function of original tissues.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval This manuscript does not have any content that requires ethical approval.

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