INTRODUCTION

Laryngeal carcinoma is the second most common head and neck cancer, and occurs more commonly in men than in women.1 With an estimated incidence rate of 5.8/100 000 in males, it can seriously threaten health and quality of life.2 Approximately 60% of patients initially present with an advanced primary tumor (stage III or IV) and, once diagnosed, usually portend a poor outcome and lower treatment efficacy. Despite continuing efforts to improve/optimize outcomes in individuals with laryngeal carcinoma and preserve laryngeal function through radiation-based strategies, there are limited therapeutic options.3-4 For advanced primary tumor(s) or previously treated dysfunctional larynx, total or partial laryngectomy plays a critical role and remains the primary method of treatment.5 These surgical methods influence the capacity of phonation and airway protection during swallowing.6 The need for laryngectomy persists among individuals with a dysfunctional larynx and poor quality of life.

Dysfunctional larynx can lead to problems with speech, breathing, swallowing, taste, and smell. However, surgery can result in significant—if not traumatic—changes to cosmetic appearance, which can be devastating to some patients. Loss of a functioning
larynx also heavily impacts social functioning and the ability to work. To address these problems, allograft transplantation of laryngeal tissue was attempted in 1969. Although the attempt was unsuccessful, it prompted more clinical research. However, to our knowledge, only two successful laryngeal allotransplantations have been reported to date. The disadvantages of laryngeal transplantation include risks for reperfusion injury and infection. Several problems, such as ethical concerns and lifelong immunosuppression, are associated with such a procedure. Moreover, functional integration with the nerve-muscle unit after allotransplantation is not currently possible.

As a rapidly expanding field, tissue engineering is reaching maturity and yielding promising outcomes. Previous research investigating tissue engineering of the larynx aims to improve functionality post-laryngectomy and reconstruct damage without the need for subsequent immunosuppression. It is more likely that restoration of laryngeal defect(s) in the future will be based on tissue engineering methods rather than allotransplantation. However, to tissue engineer a functional larynx successfully, an understanding of its normal anatomy and physiology is required.

Anatomically, the larynx is a hollow, three-dimensional structure consisting of thyroid, ring-shaped, arytenoid, and epiglottic cartilages. The cartilage in the laryngeal cavity is connected by muscles. Muscle relaxation and contraction can control the tension of the vocal cords, as well as opening and closing of the glottis. Due to its special location, function, and natural morphology, tissue engineering of the larynx presents significant challenges but also particular advantages in reconstruction. The study of cartilage tissue engineering has an important role to play in rebuilding and shaping the head and neck, as well as reconstituting, specifically in larynx cartilage reconstruction. For the past few decades, many research teams have used an excessively empirical approach to cartilage repair; however, they now tend to focus on a more biological approach using novel tissue engineering–based strategies.

In the past two decades, three key elements have formed the building blocks of the tissue engineering–based approach: a matrix scaffold, cells sources, and growth factors (or genetic regulators). The optimal tissue-engineered laryngeal cartilage with good biocompatibility and biodegradability requires a three-dimensional scaffold and a large quantity of cells and signaling molecules. The following sections describe the key constituents of a tissue engineering–based approach to laryngeal cartilage repair.

2 | CURRENT RESEARCH ACTIVITIES RELATING TO TISSUE-ENGINEERED LARYNX

2.1 | Scaffold

The first case to use a scaffold as a cell carrier in cartilage repair dates back to the 1960s. Since then, synthetic polypropylene mesh scaffolds have been used to achieve partial laryngeal replacement in pigs. To mimic the native larynx, biomaterials amenable to shaping, with specific mechanical strength, flexibility, biocompatibility, and biodegradability, are needed, not only in vitro but also in vivo. For their capacity to facilitate laryngeal cartilage reconstruction. These biomaterials can be broadly divided into two categories—natural and synthetic—which are discussed below.

Natural materials with suitable bioengineering characteristics in regulating cell response(s) include carbohydrate-based polymers (eg, polylactic acid, polyglycolic acid, hyaluronan, agarose, alginate, and chitosan) and protein-dependent polymers (eg, fibrin, gelatin, and collagen), which are generally used in cartilage repair. Some evidence supports agarose as a potential scaffold candidate because it has been used as a matrix in cartilage tissue engineering owing to its high water absorbance capacity, similar to the extracellular matrix (ECM). Similar to agarose, alginate enables maintenance of the chondrocytic phenotype and has been extensively used in tissue engineering as a cartilage substitute owing to its biocompatibility and non-immunogenicity. Many studies have demonstrated the chondrogenic potential of alginate scaffolds. Human fibrin gels, which are Food and Drug Administration-approved materials, exert a pro-inflammatory effect and induce their own degradation by components of the ECM into nontoxic endpoint components. The use of fibrin glue and chondrocytes improve the repair of cartilage in vivo. As a natural protein, collagen serves as a scaffold substitute, with good cell adhesion properties, and supports chondrocyte proliferation in vivo. Miao et al reported that collagen scaffolds can improve the process of spontaneous repair of osteochondral defects better than other hydrogels.

In addition to natural materials, synthetic materials have several potential advantages including biocompatibility, low toxicity, and excellent mechanical properties. Different types of synthetic materials are used in engineering fields, namely Dacron (polyethylene terephthalate), Teflon (polytetrafluoroethylene), carbon fiber, polyester urethane, polybutyric acid, polyethyl methacrylate, and hydroxyapatite. Polyethylene glycol is chemically synthesized to act as a supporting agent in cartilage tissue engineering with good biocompatibility and hydrophilicity. Polylactide acid (PLA) and polylactic-co-glycolic acid have been described as potential scaffold materials that promote cell proliferation and differentiation in cartilage tissue engineering. The main disadvantage of PLA is its cytotoxicity and potential to elicit immunological reactions. Although some of the listed materials are already in clinical use, most are still being tested in preclinical trials.

Using three-dimensional printing technology, the electrospinning technique and nanotechnology aim to create an absorbable and biomimetic scaffold and stimulate the extracellular microenvironment of the native cartilage. At the nanoscale level, the interaction between scaffolds and cells becomes more active owing to the unique features of nanomaterials compared with larger-scale materials. In turn, this enhances cell behavior to a significant extent, resulting in changes in cell shape and motility, along with the expression of different genes.
potential of bone marrow–derived mesenchymal stem cells (BMSCs)

However, the most significant disadvantage is that the chondrogenic
tration.42 Precursor cells of different tissue origins exist in adult mam-
imals and can be used for transplantation purposes. Mesenchymal stem cells (MSCs) are primitive precursor cells that give rise to multi-
ple cell types including osteoblasts and chondrocytes owing to their
capacity for self-renewal and accessibility.43 Other cell types, such as perinatal cells, embryonic stem cells, and chondroblasts, also have
the potential to differentiate into cartilage.44 Some notable cartilage
engineering in the field of otolaryngology includes the research by
Zhang et al, who created three-dimensional tissue-engineered la-
ryngeal cartilage from adipose-derived MSCs (ADMSCs) in vivo.40
However, the most significant disadvantage is that the chondrogenic
potential of bone marrow–derived mesenchymal stem cells (BMSCs) declines with age.45

There have been only a few studies investigating synovium-,
periodic blood-, and umbilical cord blood–derived MSCs,46 and it
remains to be confirmed whether induced pluripotent stem cells can
differentiate and mature into cartilage tissue.47 Many existing prob-
lems with stem cells, such as age, maturation state, newly formed
cells, and tissue matches with the donor, need to be resolved. The
most prominent challenge in the use of stem cells for differentiation
into chondrocytes is avoiding hypertrophy, which demands biologi-
cal, chemical, and physical regulation.48 Ongoing studies continue to
search for the ideal source of MSCs suitable for the clinical repair of
the laryngeal cartilage.

2.2 | Cell sources

Ideally, cell-based tissue-engineered laryngeal cartilage would have
cells evenly distributed throughout the scaffold, which would fuse
with the adjacent tissue (ie, laryngeal muscle) without inducing an
inflammatory response. Cell-based therapies have been shown to
repair partial laryngeal defects in vivo.40 Although autologous chon-
drocyte implantation is used to repair laryngeal cartilage defect(s)
with good results, the main drawbacks are biological and surgical
limitations.41

The search for ideal cell sources has attracted attention to the field
of cartilage regeneration as a new powerful tool in scaffold augmenta-
tion.42 Precursor cells of different tissue origins exist in adult mam-
als and can be used for transplantation purposes. Mesenchymal stem cells (MSCs) are primitive precursor cells that give rise to multi-
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In addition to creating tissue-engineered laryngeal cartilage, suc-
cessful regeneration of the laryngeal cartilage tissue not only relies on the scaffold and cells, but is also significantly influenced
by the microenvironment in which cells grow.49 Biomolecules in-
clude growth, differentiation, angiogenic, and gene-modulated
factors, which play important roles in the microenvironment.
Similar to the ECM, biomolecules have a powerful influence on
the migration, differentiation, and proliferation of cells.50,51 To
optimize differentiation, it is essential to use well-characterized
growth factors.

The main growth factors include transforming growth fac-
tor-beta (TGF-β), insulin-like growth factor-1 (IGF-1), bone morpho-
genetic proteins (BMPs), platelet-derived growth factor (PDGF),
vascular endothelial growth factor (VEGF), epidermal growth factor
(EGF), and fibroblast growth factor (FGF)-2. TGF-β is a multifunc-
tional factor in the mitogenic process that controls proliferation and
differentiation of many cell types and may enhance the activity of
PDGF, b-FGF, and EGF.55 IGF-1 has demonstrated potential in carti-
gle grafting proliferation and peripheral nerve regeneration, which
also stimulate the differentiation of MSCs in chondrogenesis.53,54
Released from activated platelets, PDGF is involved in inflammatory
responses, reconstructive processes, and hemostasis.55 It induces
collagen biosynthesis and angiogenesis as a mitogenic and che-
notactic factor.56,57 BMPs act as a key factor in osteogenesis and
osteinductively influence regeneration of the cartilage directly and
indirectly, and stimulate the differentiation of MSCs into various cell
types.58

In most cartilage-engineering strategies, many elements influ-
ence the efficacy of biomolecules, including cell stage and treat-
ment dose and duration.59 This has been evaluated mainly in vitro
and to only a limited degree in vivo (only TGF-β has been shown to
be effective).60 Nevertheless, future research will focus on testing
small signal molecules that exert a generalized anabolic effect on
chondrocytes.

| Author | country | Year | Model | Scaffold | Cell sources | Assessment | Reference |
|--------|---------|------|-------|----------|--------------|------------|-----------|
| Herrmann P et al | Britain | 2017 | Pigs | Decellularized larynx | Human BM-MSC | In vivo | [23] |
| Sun A et al | China | 2015 | Rabbits | Porous PHBHH | Costal and articular chondrocytes | In vitro | [22] |
| Gilpin DA et al | America | 2010 | Rabbits | Scaffold free | Autologous auricular chondrocytes | In vivo | [41] |
| Ansari T et al | Britain | 2017 | Pigs | Decellularized hemilarynges | Human BM-MSC | In vivo | [63] |
| Kamil SH et al | America | 2004 | Pigs | Polymer (Pluronic F-127) | Autologous auricular chondrocytes | In vivo | [24] |
| Zhang H et al | India | 2017 | Rats | Collagen oligomer | Autologous ASCs | In vivo and in vivo | [40] |
| Zetz GP et al | Brazil | 2017 | Pigs | Poly-DL-lactide | Human MSCs | In vivo | [61] |
| Omori K et al | Japan | 2008 | Human | Collagen sponge | Cell free | In vivo | [21] |
Research in laryngeal tissue engineering was hardly existent until the turn of the 21st century. The number of publications describing tissue engineering larynx has been rapid growth since that time (Table 1). Although still in its infancy, research activity investigating the application of laryngeal tissue engineering in reconstructive medicine suggests rapid advances and developments in the future. The aim of laryngeal tissue engineering is to develop methodologies by which laryngeal defects can be repaired and demonstrate its potential to transform clinical care. Although translational progress remains in the early stages, it is appropriate to assess strategic directions in laryngeal tissue engineering.

An ideal tissue-engineered constructs should mimic the internal environment and maintain the mechanical properties of the larynx. Due to special characteristics of the larynx, it is difficult to develop a tissue-engineered larynx without suitable scaffolds, cells, and growth factors (Figure 1). Most of the experience so far gained with tissue-engineered constructs has focused on biocompatible and nontoxic scaffold without cells, although cells play important roles. The "ideal" cell type must be sufficient and functional for the purpose of harvesting without infection, immune response, and possible tumor formation. This may be more technically challenging in practice. Irrespective of the type of cell that is employed, cell choice, isolation, and seeding must be concerned before the goal of larynx cartilage regeneration can be achieved. Whether to seeding remains controversial; however, the majority of research has demonstrated partiality toward seeded scaffolds. Jotz et al reported that MSC scaffolds demonstrated a significant advantage in forming laryngeal neo-cartilage in a porcine model. The seeded cells may act as a "feeder layer" by activating local progenitor cells and accelerating the process of tissue integration. Although not formally assessed, Herrmann et al demonstrated that each animal had normal respiratory, sounding, and swallowing functions post-surgery of the larynx, without adverse clinical effects due to an implanted and seeded de-cellularized scaffold.

Different from other kinds of cartilage regeneration, the construction of tissue-engineered laryngeal cartilage has intricate cartilaginous complex in keeping the airway patent demanding for biochemical and material properties. In laryngeal biology, what is needed now is a new concept of cartilage repair, cutting-edge techniques, and systematic strategies for evaluation. The implanted scaffold provides a skeletal frame for larynx regeneration. To create a suitable scaffold with a hollow structure, such as the larynx, choosing "smart" materials with excellent biocompatibility and biodegradability with minimal side effects to mimic the structure of native laryngeal cartilage is the key to improving laryngeal cartilage reconstruction.

4 | PERSPECTIVES

It is not currently possible to replace the entire larynx with fully vascularized, nerve innervated, tissue-engineered products. Laryngeal regenerative medicine is currently focused on replacing the hemilarynx as opposed to the entire larynx, while maintaining fundamental functions, such as respiratory function, in preclinical studies. Ansari et al implanted a porcine hemi-larynx into a porcine animal model to complete epithelialization of the mucosal surface without previous attempts at vascularization of the scaffold. Despite advances and rapid development in cartilage tissue engineering, functional repair using tissue-engineered laryngeal cartilage has not yet been reported in the clinic. The main problems include vascularization, mucosalization, and support in cartilage reconstruction for laryngeal cartilage. According to the common nearest transfer, using a tissue flap to provide a blood supply and subsequently preparing for the tissue-engineered cartilage may overcome the problems with vascularization. To complete mucosal coverage and heal the damaged cartilage, laryngeal replacement needs to use cells and/or growth factors to inhibit tissue scarring accompanied by inflammatory responses, including neutrophil infiltration, together with calcification.
The present review highlights the promising future of tissue-engineered laryngeal cartilage; however, there is still a need for comprehensive development of cutting-edge techniques, especially in three-dimensional printing (3D), 4D printing (3D printing of programmable inks), or 5D printing as a five-axis system for printing complex structures in multiple dimensions of tissue scaffolds for futuristic tissue engineering and regenerative medicine. A major advantage of tissue-engineered laryngeal cartilage would be the facilitated differentiation of host and donor cells. Disadvantages of grafting tissue-engineered laryngeal cartilage include the high costs and the time interval for the growth of an adequate and useable piece of cartilage. Further experimental studies and development of surgical procedures are needed to validate the use of tissue-engineered laryngeal scaffolds.

5 | CONCLUSION

Despite considerable technical obstacles, there have been rapid advances and developments in laryngeal tissue engineering. The technology of tissue-engineered larynx combined with experimental developments will improve survival and surgical outcomes in the field of laryngeal diseases. Of the proposed approaches, research focused on laryngeal studies in humans is expected in the future.

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