Received: 15 January 2020 | Revised: 6 April 2020 | Accepted: 11 May 2020

DOI: 10.1002/lio2.416

REVIEW

Laryngoscope Investigative Otolaryngology

Tissue engineering applications in otolaryngology—The state of translation

Weston L. Niermeyer MD[1] | Cole Rodman MA[2] | Michael M. Li MD[3] | Tendy Chiang MD[1,3]

[1]Department of Otolaryngology, Nationwide Children's Hospital, Columbus, Ohio

[2]The Ohio State University College of Medicine, Columbus, Ohio

[3]Department of Otolaryngology—Head and Neck Surgery, The Ohio State University Wexner Medical Center, Columbus, Ohio

Correspondence
Tendy Chiang, Department of Pediatric Otolaryngology—Head and Neck Surgery, Nationwide Children's Hospital, 700 Children's Drive, Columbus, OH 43205, USA.
Email: tendy.chiang@nationwidechildrens.org

Abstract

While tissue engineering holds significant potential to address current limitations in reconstructive surgery of the head and neck, few constructs have made their way into routine clinical use. In this review, we aim to appraise the state of head and neck tissue engineering over the past five years, with a specific focus on otologic, nasal, craniofacial bone, and laryngotracheal applications. A comprehensive scoping search of the PubMed database was performed and over 2000 article hits were returned with 290 articles included in the final review. These publications have addressed the hallmark characteristics of tissue engineering (cellular source, scaffold, and growth signaling) for head and neck anatomical sites. While there have been promising reports of effective tissue engineered interventions in small groups of human patients, the majority of research remains constrained to in vitro and in vivo studies aimed at furthering the understanding of the biological processes involved in tissue engineering. Further, differences in functional and cosmetic properties of the ear, nose, airway, and craniofacial bone affect the emphasis of investigation at each site. While otolaryngologists currently play a role in tissue engineering translational research, continued multidisciplinary efforts will likely be required to push the state of translation towards tissue-engineered constructs available for routine clinical use.

Level of Evidence: NA.

KEYWORDS

auricular, craniofacial, nasal, regenerative medicine, tissue engineering, tracheal

1 | INTRODUCTION

The evolution of reconstructive techniques has allowed for improved functional and cosmetic outcomes in head and neck surgery. Advances in local flaps and free tissue transfer demonstrate the versatility of autologous tissue, and facial transplantation has proven the viability of donor tissue for head and neck reconstruction.[1] However, limitations such as lack of donor tissue, poor tissue match, and transplant rejection persist. Tissue engineering (TE) holds the potential to address these barriers through the provision of new, healthy tissue identical to the host. The ideal tissue-engineered construct would act as an autologous replacement for diseased or surgically resected structures and possess the capacity to renew, regenerate, and repair in vivo.

Despite the vast implications generated by the Vacanti mouse bearing a human ear on its back[2] and early successes and controversies surrounding tracheal grafts,[3-6] the full potential of tissue engineering for clinical use has not been realized. In this review, we aim to appraise the current status of TE applications within otolaryngology, describe where research efforts have been focused for the past five years, and evaluate promising future directions. Further, we analyze the role of otolaryngologists within the field of regenerative medicine and describe where otolaryngologist-led work has been published.

2 | METHODS

A literature search of the PubMed database for TE articles pertaining to head and neck anatomical sites over a five-year time period spanning May 2014 to June 2019 was performed. The following searches terms were included: "tissue engineering," "regenerative medicine," "otolaryngology," "nose," "nasal," "ear," "tympanic membrane," "ossicular," "cochlea," "laryngeal," "trachea," "tracheal," "facial reconstruction," "maxillary," and "mandibular." For all results, the abstract was individually reviewed to determine that the study involved one or more components of the TE paradigm: cellular source, scaffold, and signaling—those that did not were excluded. Review and opinion articles were also excluded. For remaining results, the methods and results sections were further reviewed and those articles without a translational or clinical component (eg, those focused on biochemical pathways) were additionally excluded. Studies meeting inclusion criteria were broadly characterized into anatomic region and application and the following variables were extracted: scaffold utilization, biochemical evaluation, histological analysis, mechanical analysis, in vitro study, in vivo study, animal model, clinical outcomes, cell source, length of study, and orthotopic or heterotopic placement of experimental construct.

The role of otolaryngologists in head and neck TE was assessed through data regarding author specialty and journal of publication. Journals were classified into four categories, Basic Science, Biomedical Engineering, Medicine/Surgery, and Otolaryngology. Analyses and figures were completed using the R computing software (Version 3.6.0).

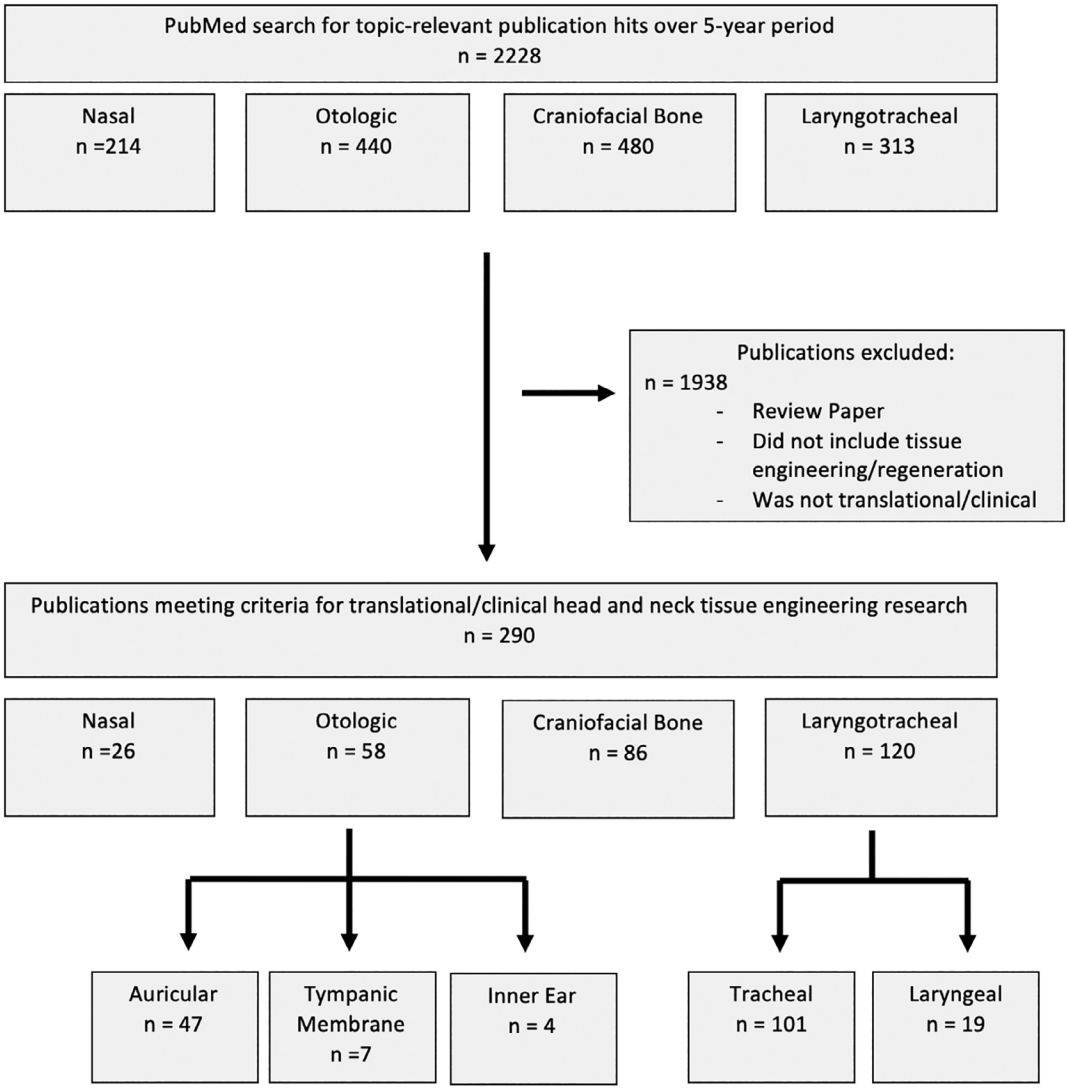

FIGURE 1 Steps taken in the determination of publications to be used for review

3 | RESULTS

After exclusion criteria were applied, a total of 290 unique peer-reviewed publications from May 2014 to June 2019 were included (Figure 1). These were anatomically characterized as laryngotracheal (41.4%, n = 120), craniofacial (29.7%, n = 86), otologic (20.0%, n = 58), and nasal (9.0%, n = 26). Publications addressed the following topics: characterization of constructs and animal model components, in vitro and in vivo studies, and small-scale human studies. The included studies most frequently involved in vitro testing, small animal models (mice and rats), or a combination of the two (Figure 2). Large animal models included rabbits, pigs, sheep, dogs, primates, monkeys, and goats. Overall, studies within TE engineering have primarily taken place in animal models, with large-scale human clinical trials yet to occur.

4 | DISCUSSION

Although overall conclusions regarding progress at each subsite can be drawn based on numerical data, each anatomic site also presents unique challenges. For example, the consequences of a failed tracheal graft may be life threatening, while that of a failed nasal graft is likely to be cosmetic only. Given this, specific details regarding each subsite are presented by anatomic region.

4.1 | Tissue engineering for nasal replacement

Nasal defects have a variety of etiologies and may cause both cosmetic and functional deficits.[7]

Currently, reconstruction with local flaps and autologous cartilage grafts remain the mainstay of therapy. However, autologous cartilage can result in donor-site morbidity and is limited in the size and shape of cartilage as well as the availability of tissue source. The lack of autologous analogues for nasal reconstruction has led to the pursuit of graft candidates using allogeneic and synthetic materials. The efficacy of these materials is well described, as are their associated complications: synthetic materials can extrude, become infected, and cause a foreign body reaction.[8,9] Likewise, allogeneic grafts are associated with immune rejection and disease transmission.[8,9] Tissue engineered cartilaginous constructs can provide tissue designed to fit the specific geometric and functional requirements of a given defect while also avoiding donor site morbidity. To achieve such a goal, successful grafts must be able to

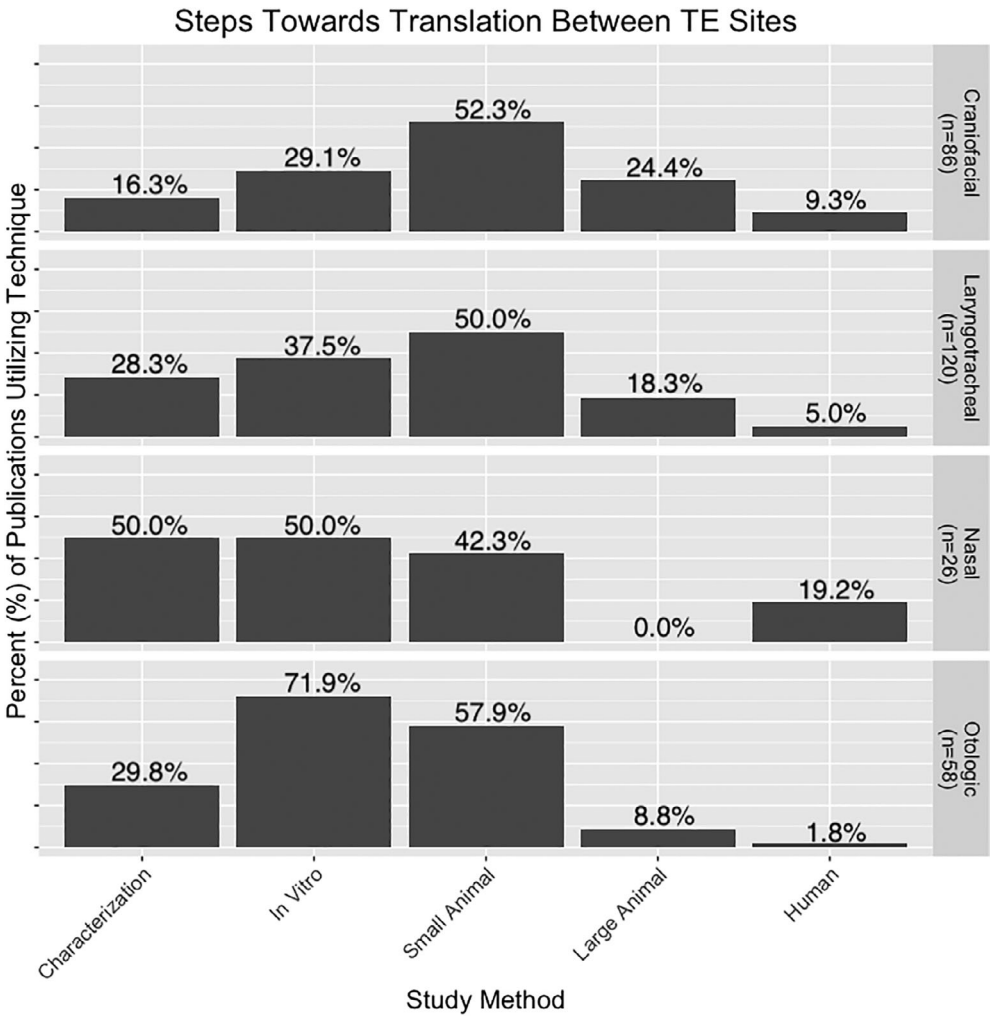

FIGURE 2 Proportion of publications including each investigation type for the head and neck TE subsites

replicate the size, shape, and mechanical properties of the nasal cartilages.[10]

Twenty-six articles were found to involve TE applications for nasal reconstruction since 2014 (Figure 3). While the total study number is relatively small, these studies present a suitable evaluation of histologic characteristics, mechanical properties, regenerative potential, and the ability to create a 3D construct using primarily in vitro and small in vivo models.[8,10-19] There has been an absence of testing in large animal models, however, there have been multiple attempts (four studies) to replace nasal cartilage subunits in humans.[9,20-22]

Utilization of human chondrocytes for cartilage regeneration has been a focus within nasal TE. Nasal septal chondrocytes, in particular, have been shown to have favorable proliferative capacity and chondrogenic potential compared to other chondrocyte sources in in vitro studies.[23,24] This tissue is easily acquired, either as remnants from septal surgery or as a biopsy of the septum with minimal donor site morbidity. Further, the ability of nasal septal progenitor cells to replicate does not diminish over prolonged cultivation, and nasal septal chondrocytes have been replicated from a small population of primary cells without the need for a scaffold.[23,24] Such cartilaginous tissue-engineered grafts have successfully been utilized in human subjects. Fulco et al used autologous nasal septal chondrocytes seeded on collagen membranes to reconstruct two layer alar lobule defects following tumor resection in five patients.[9] At one year, patients were satisfied with functional and aesthetic outcomes. Hoshi et al utilized a collagen scaffold-based tissue-engineered cartilage to augment the nasal dorsum in patients with cleft lip-nose deformity. Patients experienced improved nasal shape and a clinical trial was initiated for further investigation.[21]

The complex and varied geometry of nasal cartilage has driven the use of three-dimensional printing and injectables in scaffold construction. Xu et al used three-dimensional printing to replicate lower lateral nasal cartilages that were subsequently grown subcutaneously in mice. This resulted in a precise construct that possessed morphologic features similar to the native cartilage, but with greater biomechanical strength.[10] Other studies have used injectable autologous nasal chondrocyte and platelet-rich plasma grafts to treat external nasal valve collapse in humans.[20,22] This minimally invasive approach has proven especially valuable in the setting of insufficient structural support without a major soft tissue defect.

Overall, the subset of nasal TE publications in the past five years is small but includes the highest proportion of human studies. (Figure 3). It is likely that the simplicity and low morbidity of cartilage-only nasal grafts has created the opportunity for immediate investigation in human patients.[9,20-22] The promising results from these studies have enabled investigators to initiate movement toward larger-scale trials.[9] Meanwhile, no studies have been completed demonstrating creation of a true composite graft including nasal mucosa, cartilage,

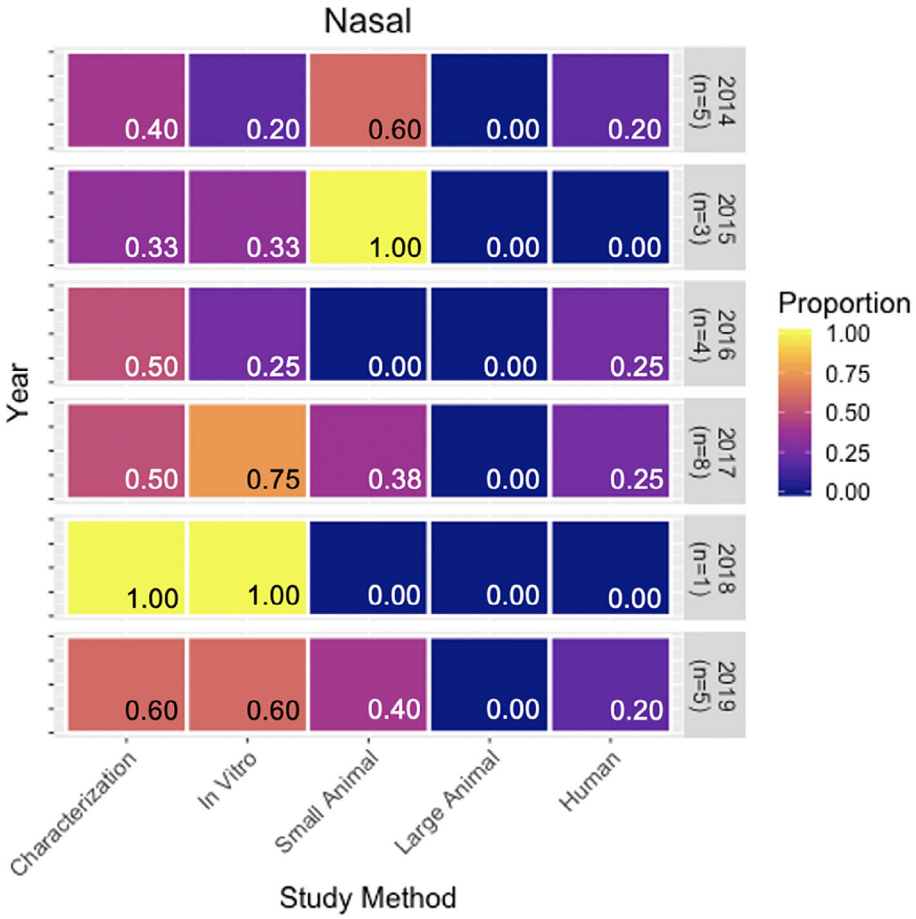

FIGURE 3 Heatmap representing the proportion of publications by year that utilized specific translational research methodologies from construct characterization to human trial to investigate nasal tissue engineering[8-24,98-106]

soft tissue, and overlying skin which would be able to serve as a reconstructive option in a full-thickness nasal defect.

4.2 | Regenerative medicine for otologic applications

TE applications for the ear have focused primarily on auricular reconstruction with limited investigation into regeneration of inner and middle ear structures. In particular, various stem cell and scaffold materials have been used to develop constructs to regenerate tympanic membrane perforations.[25-31] Other work has focused on in vitro models of decellularized cochleae and the establishment of pluripotent stem cell lines with the goal of generating functional inner ear hair cells.[32-35] While ossicular chain tissue engineering has been investigated in the past, there were no new reports within our inclusion.[36,37] Thus, the majority of efforts in otologic applications in TE have been focused on cartilaginous reconstruction of the auricle. Currently, reconstructive efforts rely on protheses or autologous cartilage grafts. However, a prosthesis can extrude and, although biocompatible, cartilage autografts increase donor site morbidity and may not provide optimal size and function match. Reconstruction of the external ear is a technically challenging, surgeon dependent, multistage procedure which requires multiple costal cartilage segments to be harvested, putting the patient at repeated risk of donor site morbidity.[38] Finally, the arrangement of cartilage can warp over time and lead to poor long-term outcomes. TE offers an alternative source of autologous cartilage that is not limited by quantity or shape and can be used to improve auricular reconstruction.

A total of 58 articles in the past five years have focused on the development of otologic TE constructs, with 47 of these examining auricular TE specifically. Efforts to develop auricular TE constructs have focused on the combined use of in vitro and in vivo models, and only one human study was published during the review period (Figure 4). The majority of investigations have been focused on improving construct flexibility and preventing contraction in vivo through heterotopic or orthotopic placement.

Decellularized cartilage provides a scaffold that acts as an organized microenvironment for cartilage TE. While demonstrated in other subsites, not until 2015 had a group demonstrated that human bone marrow-derived mesenchymal stem cells seeded onto decellularized auricular cartilage were able to differentiate in vitro.[39] Further work aimed at optimizing the histologic and biomechanical properties of TE constructs has shown that culture under dynamic conditions prior to implantation into a mouse model is beneficial.[40]

Other groups have investigated the use of hydrogels—hydrophilic three-dimensional polymeric networks that swell in water.[41] Hydrogels possess high water content and elasticity, making them better

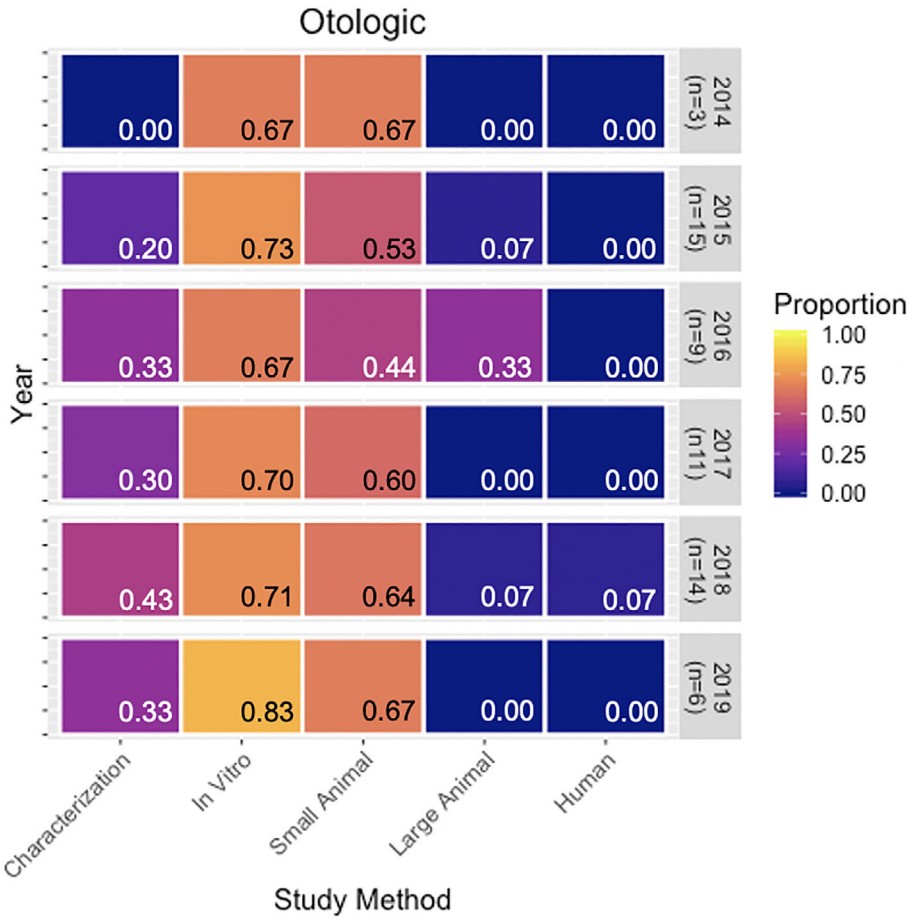

FIGURE 4 Heatmap representing the proportion of publications by year that utilized specific translational research methodologies from construct characterization to human trial to investigate otologic tissue engineering[15,25-35,38-43,45,46,107-144]

mimics of human tissue than other synthesized materials. Investigations into hydrogels have sought to optimize the ratio of materials used to comprise the gels, and have shown their cartilage-forming potential through in vitro and in vivo studies.[41-43] Of particular interest, hydrogels can be combined with 3D-printed molds to form a TE construct that better recapitulates the native auricle.

When a costal cartilage graft is used to reconstruct the auricle, the cartilage can calcify, causing it to thicken and deform.[44] Visscher et al addressed this problem by adding both a 3D-printed poly-e-caprolactone mold and collagen scaffold to a cell-seeded hydrogel, and demonstrated creation of contraction free constructs in vitro.[43] Others have shown longer in vitro culture of a construct prior to in vivo implantation reduces contraction in vivo.[40] Pomerantseva et al utilized a sheep model to demonstrate that auricular chondrocytes could be expanded to a quantity needed for a whole auricle and that the overall shape of the engineered ear could be preserved with minimal dimensional changes.[45]

As with nasal TE, the majority of auricular TE research has primarily been conducted in in vitro and animal models without a trend towards human studies published in the last five years. The first report of clinical auricular TE came in 2018: five children with microtia between the ages of 6 and 9 were treated with a patient-specific ear-shaped engineered cartilage.[46] Investigators expanded harvested microtia chondrocytes, seeded these on a 3D-printed biodegradable scaffold, and cultured the construct in vitro. Patients underwent tissue expansion prior to reconstruction and were followed for 2.5 years postimplantation, with satisfactory aesthetic outcomes reported.[46] While promising, follow up studies have not been performed, and there have not been large-scale clinical trials. Future studies should work to optimize construct materials and the process of auricular reconstruction using TE further to make such products clinically available on a larger scale.

4.3 | Tissue engineering for craniofacial reconstruction

Craniofacial reconstruction allows for the restoration of facial symmetry and functional architecture for speech, breathing, and mastication. Current reconstruction utilizes bone grafting, which is limited to smaller defects, and the osteocutaneous free flap (OCFF), whose versatility permits reconstruction of some larger defects.[47] However, osteocutaneous free tissue transfer does not fully recapitulate the complexity and dimensions of craniofacial bone. Further, OCFF is a highly complex surgery requiring microvascular surgeons and donor site harvesting that can be associated with significant morbidity. Regenerative medicine offers an alternative to free tissue transfer for addressing large craniofacial bone defects that eliminates the need for microvascular surgery and the possibility of donor site morbidity.

A successful scaffold for craniofacial bone repair needs to replicate osteoconduction, osteogenesis, and osteoinduction to repair bony defects. Nonautologous bone graft substitutes have been used to make scaffolds, and one area of continued investigation has been the optimization of these scaffolds' structural properties. Commonly used substitutes are hydroxyapatite, calcium carbonate, demineralized bone matrix (DBM), and beta-tricalcium phosphate[48-58] Three-dimensional printing has the potential to play an important role in customized defect repair and head and neck bone TE and is in the early stages of in vivo investigation. Lopez et al treated mandibular defects in a rabbit model with a 3D-printed bioceramic scaffold that exhibited bony ingrowth.[59] 3D-printing has also been able to generate microstructures that simulate the stiffness of the mandibular condyle and even have demonstrated compressive resistances 15 times greater than bone in a rabbit model.[54,60]

Building upon construct scaffolding, the role of construct seeding with stem cells to supplement osteogenesis has been actively studied. Mesenchymal stems cells (MSCs) have been shown to exhibit osteoprogenitor differentiation, osteoblast proliferation, and matrix deposition in vitro and in vivo.[57,61-66] Furthermore, the study of growth signaling has become important for growth of alveolar, maxillary, and mandibular TE constructs. A plethora of graft biomaterials and biochemical factors have been studied with the goal of improving the tissue-engineered construct's scaffold integration and tissue growth.[48-58,66-79]

Overall, craniofacial tissue engineering appears to be slightly more robust than the burgeoning work in nasal and auricular TE (Figure 5). Over the past five years, small and large animal in vivo models have been utilized to investigate TE applications for craniofacial bone, with a number of human studies having been completed as well (Figure 5). Seventy-four (86.0%) of the 86 studies reviewed investigated constructs in animals, and 8 (9.3%) of these studies investigated constructs in human patients. Specific challenges to this subsite relate to the size and location of facial bone defects with large bone TE constructs struggling to achieve adequate cell penetration and vascularization. Approaches to overcoming these challenges include the use of injectable constructs and in vivo bioreactors. Song et al developed a cell-laden hydrogel microfiber-injectable scaffold that was delivered to a defect and maintained cell viability with more even distribution than a rigid scaffold in a rat model. The cell encapsulating microfibers quickly degraded and released cells, which led to a new bone area fraction that was greater than threefold that of the control construct.[56]

In vivo bioreactors have been utilized to create autologous bone flaps for craniofacial defects.[80] The vascularization and precursor cells present in these bioreactors allow for larger bone constructs to be engineered.[81] The first human case of mandibular reconstruction using the greater omentum as a bioreactor was reported in 2016.[82] A follow-up investigation after 10 months showed that the amount of vital mineralized bone tissue of the graft in the mandible had continued to increase.[81] A morselized bone autograft was utilized as the scaffold in an in vivo bioreactor study that successfully replaced angle of mandible defects in sheep.[83] By using an in vivo autologous tissue construct, biocompatibility is promoted and the risk of dehiscence, such as has been seen with titanium mesh, can be mitigated.[81] It has also since been shown that dental implants are able to be

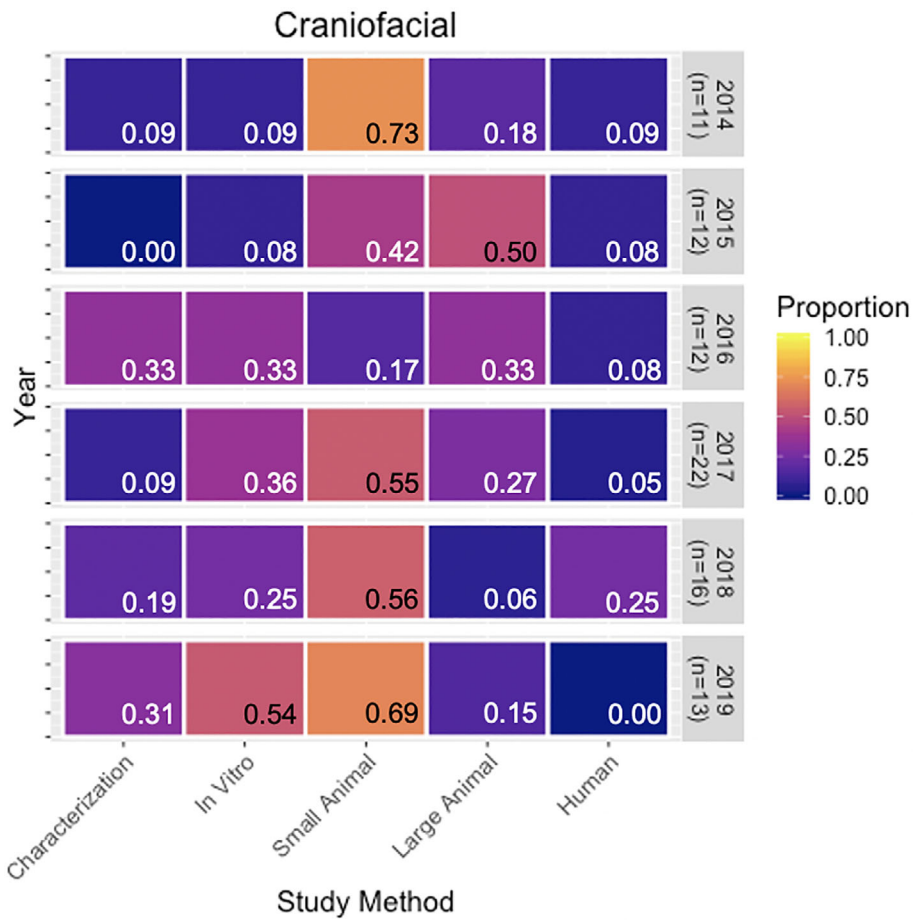

FIGURE 5 Heatmap representing the proportion of publications by year that utilized specific translational research methodologies from construct characterization to human trial to investigate craniofacial tissue engineering[49-72,74-77,79,81-84,145-197]

osseointegrated into de novo tissue engineered bone in an animal model that utilized an in vivo bioreactor.[84]

Despite these promising options for addressing large bone defects, segmental defects of the mandible have not been investigated thoroughly. The bulk of studies have instead focused on small bone defects, with only one actually removing an entire segment of mandible. The researchers used a biodegradable scaffold in a monkey model and found that it had insufficient load bearing capacity and incomplete bone unity after 6 months.[52] Since the mandible is the seat of dentition, and plays a role in speech, mastication, and facial appearance, it is important to work toward an engineered construct that can repair such segmental mandibular defects.

4.4 | Regenerative medicine for laryngotracheal replacement

The majority of work in laryngotracheal TE has occurred in tracheal replacement, with limited work being performed in the larynx as well. Broadly, laryngeal TE currently seeks to restore respiratory and vibratory function. Maintenance of the neuromuscular connections that are required for function of the larynx is a specific challenge. Brookes et al found that rats with recurrent laryngeal nerve injury had improved muscle recovery after treatment with TE motor endplate constructs rather than primary muscle progenitor cells alone.[85] Small and large animal in vivo models have been used to show that stem cell-seeded constructs are able to produce sufficiently mucosalized vocal folds.[86,87] Hermann et al produced rudimentary vocal folds with appropriate mucosal coverage in pigs such that the animals were able to maintain aeration, phonation, and swallowing.[87]

Tracheal TE research has primarily focused on reconstructing long-segment defects (>50% in adults and >30% in children). These may arise from either congenital or acquired etiologies and require tissue transfer or implantation to reconstruct.[88] Early excitement regarding tissue-engineered tracheal grafts (TETGs) was driven in part by reports of successful implantation of TE constructs in humans in 2008.[89,90] However, these early subjects suffered significant morbidity, and in some cases, mortality, leading to renewed interest in small animal and in vitro approaches. As with other sites, current and historical reconstructive efforts have focused on autologous free-tissue transfer, biomimetics made of foreign materials, transplantation and combinations of these.[88] Unsurprisingly, such approaches are limited by inadequate tissue, nonhomologous tissue, graft rejection, infection, and tissue extrusion. A successful TETG requires a biomechanically equivalent cartilaginous construct lined by a fully functioning respiratory epithelium.

Recently, in vivo models have been regularly used to develop tracheal tissue engineering constructs (Figure 6). In the past five years

alone, 120 laryngotracheal articles have been published. Of the 101 articles focusing specifically on tracheal reconstruction, 72.7% have used an animal model. Investigations have examined the ideal scaffold material with studies evaluating decellularized tracheal scaffolds, biosynthetics, and scaffold-free constructs. Similarly, the ideal cellular source for graft seeding is being pursued. Recent efforts have been devoted to addressing the predominant barriers to translation: delayed graft epithelialization, host inflammatory response and graft stenosis.

Commonly used scaffold materials include decellularized tissue, poly-lactic-co-glycolic acid (PLGA), poly-ε-caprolactone (PCL), polyethylene terephthalate (PET), and polyurethane (PU). Constructs have been created successfully with each of these, but no definitive answer regarding the best scaffold material has been achieved. Maughan et al compared allografts, decellularized allografts, and synthetic scaffolds in rabbits and did not identify one superior choice as each material was limited by a combination of inflammation, mucus plugging, lack of angiogenesis, or stenosis.[91] Further, few studies have examined the biomechanical properties of each scaffold material. Zhao et al demonstrated graft tensile and compressive strength greater than that of native trachea when using a seeded and subsequently decellularized stent with the scaffold comprised of polyglycolic acid and metal.[92] Dharmadhikari et al compared nonresorbable and resorbable scaffolds and found that both scaffolds held greater tensile strength than native trachea with nonresorbable scaffold being stiffer than resorbable.[93] However, both scaffold types were complicated by stenosis when implanted in mice with resorbable scaffolds demonstrating tracheomalacia and nonresorbable showing tissue overgrowth. Interestingly, in resorbable scaffolds, greater scaffold cellular infiltration correlated with improved survival. To that end, Best et al compared scaffold properties with different ratios of PET and PU spun onto either solid or porous C-shaped polycarbonate rings.[94] While both solid and porous rings provided excellent scaffold strength, cell seeding was superior in the solid ring construct.

Similar to the question of scaffold material, the ideal cellular source for graft seeding has not been elucidated. Various groups have experimented with an array of cellular material including epithelial cells, fibroblasts, septal chondrocytes, adipose derived stem cells (ADSCs), and bone marrow derived mesenchymal stem cells (BM-MSCs). Regardless of the cellular source, the goal of cell seeding is to create a scaffold with terminally differentiated chondrocytes and respiratory epithelium. When seeding decellularized scaffolds, Go et al showed that both epithelial and mesenchymal stem cells are necessary for graft function.[95] It has also been shown that seeded ADSCs differentiated into stromal cells, chondrocytes, and epithelial cells.[96]

Graft stenosis has plagued implanted scaffolds despite variations in scaffold and seeding material. In a recent study by Pepper et al, scaffolds were implanted in eight sheep with all eight subjects going

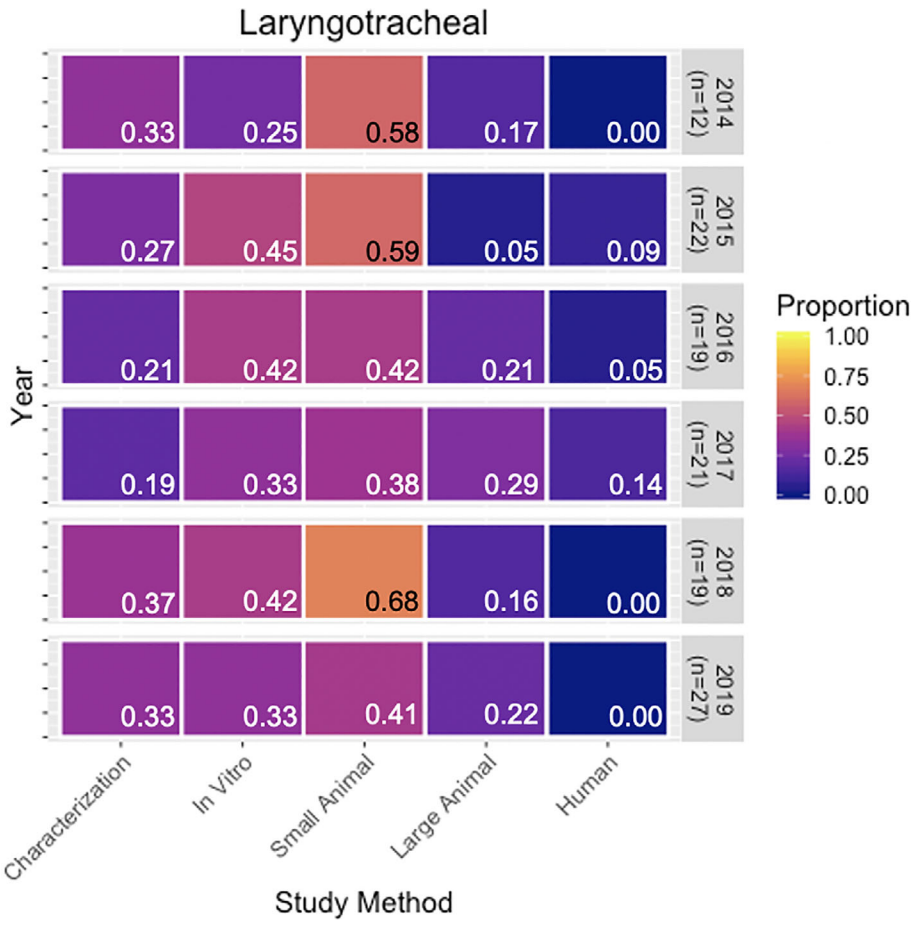

FIGURE 6 Heatmap representing the proportion of publications by year that utilized specific translational research methodologies from construct characterization to human trial to investigate laryngeal tissue engineering[85-87,91,92,94,96,97,198-306]

on to develop graft stenosis.[97] Inflammatory complications were demonstrated in the acute and chronic settings with fibrinopurulent exudate seen at postoperative day 1 bronchoscopy in all eight subjects. Finally, none of the eight scaffolds were shown to have epithelial lining at the planned euthanasia timepoint of four months. Given the aforementioned function of the epithelium in innate immunity and ciliary clearance, it is clear that the lack of epithelization is contributing to a proinflammatory response creating stenosis and chronic inflammation in tissue engineered trachea. Further studies regarding the mechanisms of graft epithelialization are critical.

Again, as with the other subsites, the majority of studies in the last five years have occurred in animal models without a significant trend towards large animal or human studies. Limitations at the tracheal subsite are uniquely related to the fact that the orthotopic implantations are not perfused well and are subject to contamination and infection. This adds an additional layer of variability in an already complex system in which scaffold material (including the ratios of said materials) and cellular source already represent sources of variation. Heterotopic implantations have introduced a stage of neovascularization that may improve TETG outcomes. Thus, the ultimate result of a TETG is dependent on exogenous and endogenous factors. Regardless, given that bioequivalent mechanical strength has been demonstrated through various methodologies, it may be that graft epithelialization and neovascularization stand to be the most important areas to address in tracheal tissue engineering moving forward.

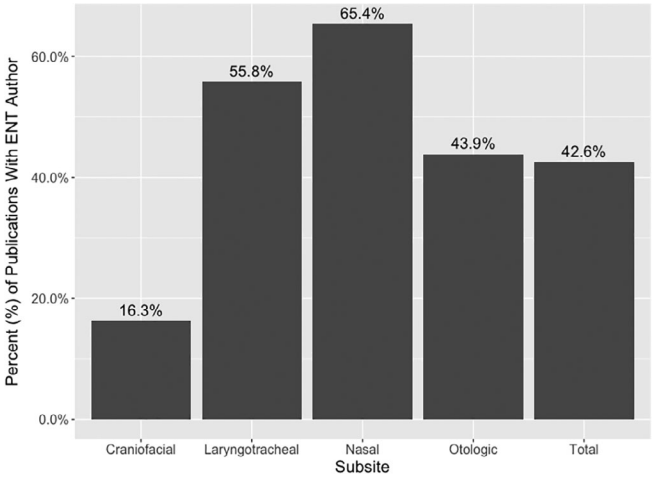

FIGURE 7 Percent of publications that include one or more otolaryngologist author

4.5 | Otolaryngologists in tissue engineering

To further explore the role of otolaryngologists in tissue engineering of the head and neck, we examined the credentials of the authors of the publications reviewed in our literature search. Of the 290 total publications reviewed, 42.6% included at least one otolaryngologist author (Figure 7). However, otolaryngology journals represent only 17% of all publications, compared to 36% of publications in biomedical engineering journals, 29% in Medicine and Surgery journals, and 17% in Basic Science journals (Figure 8). While otolaryngologists are taking an active role in head and neck applications for tissue engineering, such work has not been published in otolaryngologic journals—a disparity that may influence the exposure of the field of regenerative medicine to our colleagues. This distribution is likely influenced by the

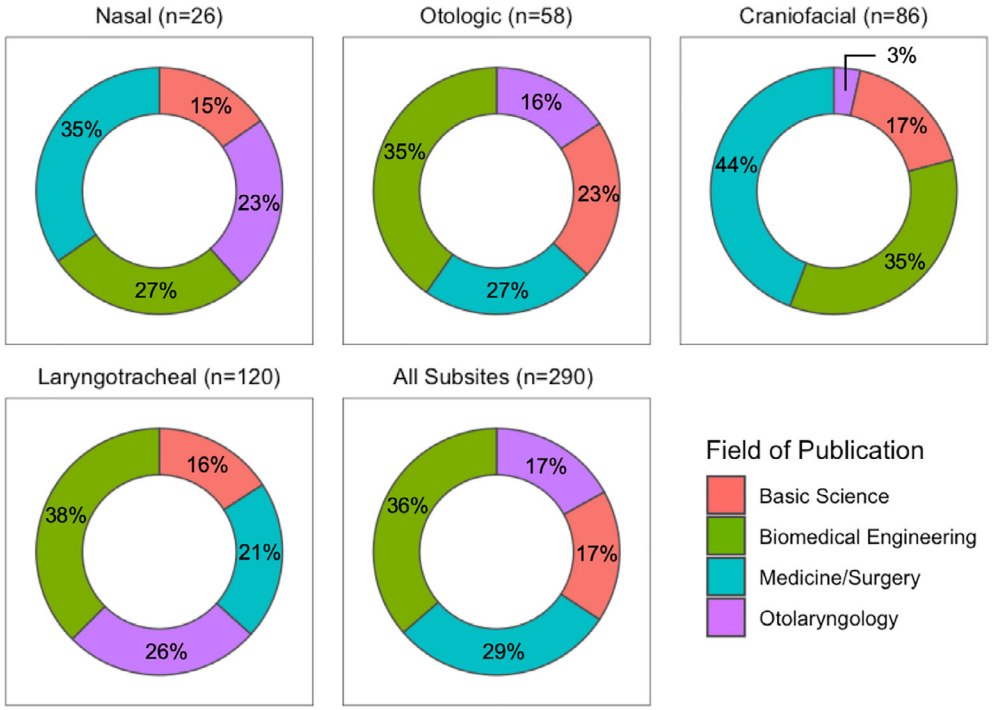

FIGURE 8 Breakdown of head and neck TE application publications by type of journal

fact that the majority of work remains in in vivo and animal models. As the number of TE companies in the United States continues to grow and engineered materials for the head and neck become more clinically available, it is to be expected that the role of otolaryngologists in the field will continue to grow.

5 | CONCLUSION

Tissue engineering holds the potential for reconstruction with autologous tissue that is not limited by availability of patient donor site tissue. The external ear, nose, trachea, and facial skeleton are important to human function and appearance and stand to benefit greatly from TE constructs. However, these subsites vary in their makeup and require individualized investigation to develop the appropriate TE construct. While the common goal of regenerative medicine is to create a construct for human use, current work in all major head and neck subsites has mostly been limited to in vitro and animal models. Throughout the last five years, there has not been a substantial shift in the proportion of TE studies that have been completed in large animal or human models. Finally, otolaryngologists participate in a significant proportion of TE studies, with work being published in a diverse range of basic science and otolaryngology-focused journals. Future studies in the field should be guided by and build upon previously completed work in an effort to move towards large animal and human models.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ORCID

Weston L. Niermeyer https://orcid.org/0000-0001-7580-8731
Michael M. Li https://orcid.org/0000-0002-0229-7667
Tendy Chiang https://orcid.org/0000-0003-1448-5873

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How to cite this article: Niermeyer WL, Rodman C, Li MM, Chiang T. Tissue engineering applications in otolaryngology—The state of translation. *Laryngoscope Investigative Otolaryngology*. 2020;5:630–648. https://doi.org/10.1002/lio2.416