Tissue engineering applications in otolaryngology—The state of translation

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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. 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 and early successes and controversies surrounding tracheal grafts, 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.⁸¹⁰⁻¹⁹ 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.⁸⁻¹⁹,²⁰⁻¹⁰⁶
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. 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. While ossicular chain tissue engineering has been investigated in the past, there were no new reports within our inclusion. The majority of efforts in otologic applications in TE have been focused on cartilaginous reconstruction of the auricle. Current 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. 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. 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. Other groups have investigated the use of hydrogels—hydrophilic three-dimensional polymeric networks that swell in water. Hydrogels possess high water content and elasticity, making them better...
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 stem cells (MSCs) have been shown to exhibit osteogenitor 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
osseointegrated into de novo tissue engineered bone in an animal model that utilized an in vivo bioreactor.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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, bio-synthetics, 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. 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. 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. 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. 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. It has also been shown that seeded ADSCs differentiated into stromal cells, chondrocytes, and epithelial cells.

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 through the procedure. The heatmap below (Figure 6) represents 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

**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. 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.

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
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.

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REFERENCES

1. Giatsidis G, Sinha I, Pomahac B. Reflections on a decade of face transplantation. Ann Surg. 2017;265:841–846.
2. Cao Y, Vacanti J, Paige K, Upton J, Vacanti C. Transplantation of chondrocytes utilizing a polymer-cell construct to produce tissue-engineered cartilage in the shape of a human ear. Plast Reconstr Surg. 1997;100:297–302.
3. Warren M. U.K. trials of airway transplants are in limbo. Science. 2018;359:1448–1450.
4. Fountain H, Christopher Lyles, got synthetic trachea, dies at 30. New York Times. 2013.
5. Fountain H. Young girl given bioengineered windpipe dies. New York Times. 2013.
6. Fountain H. Groundbreaking surgery for girl born without windpipe. New York Times. 2013.
7. Austin GK, Shockley WW. Reconstruction of nasal defects: contemporary approaches. Curr Opin Otolaryngol Head Neck Surg. 2016;24(5):453–460. https://doi.org/10.1097/MOO.0000000000000295
8. Kushnaryov A, Yamaguchi T, Briggs K, et al. Evaluation of autogenous engineered septal cartilage grafts in rabbits: a minimally invasive preclinical model. Otolaryngol Neck Surg. 2013;149(2 suppl):P37–P38. https://doi.org/10.1177/0194599813495815a17
9. Fulco I, Miot S, Haug MD, et al. Engineered autologous cartilage tissue for nasal reconstruction after tumour resection: an observational first-in-human trial. Lancet. 2014;384(9940):337–346. https://doi.org/10.1016/S0140-6736(14)60544-4
10. Xu Y, Fan F, Kang N, et al. Tissue engineering of human nasal alar cartilage precisely by using three-dimensional printing. Plast Reconstr Surg. 2015;135(2):451–458. https://doi.org/10.1097/PRS.0000000000000856
11. Gupta N, Cruz MA, Nasser P, Rosenberg JD, latridis JC. Fibrinogenipin hydrogel for cartilage tissue engineering in nasal reconstruction. Ann Otol Rhinol Laryngol. 2019;128(7):640–646. https://doi.org/10.1177/0003484919836667
12. Dufour A, Buffier M, Vertu-Colino D, Disant F, Mallein-Gerin F, Perrier-Groult E. Combination of bioactive factors and IEIK13 self-assembling peptide hydrogel promotes cartilage matrix production by human nasal chondrocytes. J Biomed Mater Res A. 2019;107(4):893–903. https://doi.org/10.1002/jbma.36612
13. Vedicherla S, Buckley CT. Rapid chondrocyte isolation for tissue engineering applications: the effect of enzyme concentration and temporal exposure on the matrix forming capacity of nasal derived chondrocytes. Biomed Res Int. 2017;2017:1–12. https://doi.org/10.1155/2017/2395138
14. Andrews SHJ, Kunze M, Mulet-Sierra A, et al. Strategies to mitigate variability in engineering human nasal cartilage. Sci Rep. 2017;7(1):1–11. https://doi.org/10.1038/s41598-017-06666-2
15. Akbari P, Waldman SD, Cushing SL, et al. Bioengineering pediatric scaffold-free auricular cartilaginous constructs. Laryngoscope. 2017;127(5):E153–E158. https://doi.org/10.1002/lary.26395
16. von Bonhard A, Elsaesser A, Riepl R, et al. Cartilage regeneration using decellularized cartilage matrix: long-term comparison of subcutaneous and intranasal placement in a rabbit model. J Cranio-Maxillofac Surg. 2019;47(4):682–694. https://doi.org/10.1016/j.jcims.2019.01.010
17. Yi HG, Choi YJ, Jung JW, et al. Three-dimensional printing of a patient-specific engineered nasal cartilage for augmentative rhinoplasty. J Tissue Eng. 2019;10:1–14. https://doi.org/10.1177/2041731418824797
18. Mendelson A, Ahn J, Paluch K, Embree M, Mao J. Engineered nasal cartilage by cell homing: a model for augmentative and reconstructive rhinoplasty. Plast Reconstr Surg. 2014;133(6):1343–1354. https://doi.org/10.1038/jpd.2014.371
19. Stuart MP, Matsui RAM, Santos MFS, et al. Successful low-cost scaffold-free cartilage tissue engineering using human cartilage progenitor cell spheroids formed by micromolded nonadhesive hydrogel. Stem Cells Int. 2017;2017:1–11. https://doi.org/10.1155/2017/7053465
20. Ceccarelli G, Gentile P, Marcarelli M, et al. In vitro and in vivo studies of alar-nasal cartilage using autologous micro-grafts: the use of the Rigenera® protocol in the treatment of an osteochondral lesion of the nose. Pharmaceuticals. 2017;10(2):1–10. https://doi.org/10.3390/ph10020053
21. Hoshi K, Fujihara Y, Saijo H, et al. Three-dimensional changes of noses after transplantation of implant-type tissue-engineered cartilage for secondary correction of cleft lip–nose patients. Regen Ther. 2017;7:72–79. https://doi.org/10.1016/j.reth.2017.09.001
22. Gentile P, Scioli MG, Bielli A, Orlandi A, Cerovelli V. Reconstruction of alar nasal cartilage defects using a tissue engineering technique based on a combined use of autologous chondrocyte micrografts and platelet-rich plasma: preliminary clinical and instrumental evaluation. Plast Reconstr Surg – Glob Open. 2016;4(10):1–7. https://doi.org/10.1097/GOX.00000000000001027
23. Kim DH, Lim JY, Kim SW, et al. Characteristics of nasal septal cartilage-derived progenitor cells during prolonged cultivation. Otolaryngol – Head Neck Surg. 2018;159(4):774–782. https://doi.org/10.1177/0194599818777195
24. Chiu LLY, To WTH, Lee JM, Waldman SD. Scaffold-free cartilage tissue engineering with a small population of human nasoseptal chondrocytes. Laryngoscope. 2017;127(3):E91-E99. https://doi.org/10.1002/lary.26396

25. Kim SW, Kim J, Seonwoo H, et al. Latent progenitor cells as potential regulators for tympanic membrane regeneration. Sci Rep. 2015;5:1-8. https://doi.org/10.1038/srep11542

26. Seonwoo H, Shin B, Jung KJ, et al. Stem cell-stimulating therapy for regeneration of chronic tympanic membrane perforations using IGF-FR2-releasing chitosan patch scaffolds. J Biomater Appl. 2019;34(2):198-207. https://doi.org/10.1177/0885328219845082

27. Zhang WJ, Ming LG, Sun JJ. Epithelial defect repair in the auricle and auditory meatus by grafting with cultured adipose-derived mesenchymal stem cell aggregate-extracellular matrix. Chin Med J (Engl). 2019;132(6):680-689. https://doi.org/10.1097/CMAH.00000000000000125

28. Kozin ED, Black NL, Cheng JT, et al. Design, fabrication, and in vitro testing of novel three-dimensionally printed tympanic membrane grafts. Hear Res. 2016;340:191-203. https://doi.org/10.1016/j.heares.2016.03.005

29. Mota C, Danti S, D’Alessandro D, et al. Multiscale fabrication of bio-mimetic scaffolds for tympanic membrane tissue engineering. Biofabrication. 2015;7(2):1-21. https://doi.org/10.1088/1758-5090/7/2/020055

30. Jang CH, Ahn SH, Lee JW, Lee BH, Lee H, Kim GH. Mesenchymal stem cell-laden hybrid scaffold for regenerating subacute tympanic membrane perforation. Mater Sci Eng C. 2017;72:456-463. https://doi.org/10.1016/j.msec.2016.11.094

31. Mellott AJ, Shinogle HE, Nelson-Brantley JG, Detamore MS, Staecker H. Exploiting decellularized cochlear as scaffolds for inner ear tissue engineering. Stem Cell Res Ther. 2017;8(1):41. https://doi.org/10.1186/s13287-017-0505-6

32. Mellott AJ, Detamore MS, Staecker H. The use of human Wharton’s jelly cells for cochlear tissue engineering. Methods Mol Biol. 2016;1427:319-345. https://doi.org/10.1007/978-1-4939-3615-1_19

33. Neal CA, Nelson-Brantley JG, Detamore MS, Staecker H, Mellott AJ. A protocol for decellularizing mouse cochlea for inner ear tissue engineering. J Vis Exp. 2018;2018(131). https://doi.org/10.3797/56523

34. Koehler K, Nie J, Longworth-Mills E, et al. Generation of inner ear organoids with functional hair cells from human pluripotent stem cells. Nat Biotechnol. 2017;35(6):583-589. https://doi.org/10.1038/nbt.3575. Systems

35. Sun JI, Li XS. A study on reconstruction of ossicular chain by an in situ bone tissue engineering technique. Acta Otolaryngol. 2009;129(5):507-511. https://doi.org/10.1007/s00405-006-4080-2

36. D’Alessandro D, Danti S, De Vito A, Forli F, Bruschini L, Berrettini S. Histologic characterization of human ear ossicles for the development of tissue-engineered replacements. Otol Neurotol. 2012;33(8):1458-1468. https://doi.org/10.1097/MAO.0b013e31826ea527d

37. Griffin M, Kalaskar D, Butler P. Argon plasma modified nanocomposite polyurethane scaffolds provide an alternative strategy for cartilage tissue engineering. J Nanobiotechnol. 2019;17(1):1-13. https://doi.org/10.1186/s12951-019-0477-z

38. Utomo L, Pleumeekers MM, Nimeskern L, et al. Preparation and characterization of a decellularized cartilage scaffold for ear cartilage reconstruction. Biomed Mater. 2015;10(1):1-11. https://doi.org/10.1088/1748-6041/10/1/015010

39. Zhao X, Bichara DA, Zhou L, et al. Conditions for seeding and promoting neo-auricular cartilage formation in a fibrosus collagen scaffold. J Cranio-Maxillofac Surg. 2015;43(3):382-389. https://doi.org/10.1016/j.jcms.2014.12.007

40. Lee JM, Suman MT, Kim SH, et al. Artificial auricular cartilage using silk fibroin and polyvinyl alcohol hydrogel. Int J Mol Sci. 2017;18(8):1707-1721. https://doi.org/10.3390/ijms18081707

41. Xiao IA, Levato R, Webb WR, Khan IM, Breugem CC, Malda J. Progenitor cells in auricular cartilage demonstrate cartilage-forming capacity in 3D hydrogel culture. Eur Cells Mater. 2018;35:132-150. https://doi.org/10.22203/ectm.v03s10

42. Visscher DO, Gledaill A, Busheron J, et al. Design and fabrication of a hybrid alginate hydrogel/poly(lactic-co-glycolic) mold for auricular cartilage reconstruction. J Biomed Mater Res B: Appl Biomater. 2019;107(5):1711-1721. https://doi.org/10.1002/jbm.b.34264

43. Jessop ZM, Javed M, Otto IA, et al. Combining regenerative medicine strategies to provide durable reconstructive options: auricular cartilage tissue engineering. Stem Cell Res Ther. 2016;7(1):1-12. https://doi.org/10.1186/s13287-015-0273-0

44. Pomerantsseva I, Bichara DA, Tseg A, et al. Ear-shaped stable auricular cartilage engineered from extensively expanded chondrocytes in an immunocompetent experimental animal model. Tissue Eng A. 2016;22(3-4):197-207. https://doi.org/10.1089/ten.tea.2015.0173

45. Zhou G, Jiang H, Yin Z, et al. In vitro regeneration of patient-specific ear-shaped cartilage and its first clinical application for auricular reconstruction. EBioMedicine. 2018;28:287-302. https://doi.org/10.1016/j.ebiom.2018.01.011

46. Fishero B, Kohli N, Das A, Christopher J, Cui Q. Current concepts of bone tissue engineering for craniofacial bone defect repair. Cranio Maxillofac Trauma Reconstr. 2014;08(01):023-030. https://doi.org/10.1005/i.s003-1393724

47. Liang F, Leland H, Jedrezewski B, et al. Alternatives to autologous bone graft in alveolar cleft reconstruction: the state of alveolar tissue engineering. J Craniofac Surg. 2018;29(3):584-593. https://doi.org/10.1097/SCS.0000000000004300

48. Fan J, Park H, Lee MK, et al. Adipose-derived stem cells and BMP-2 delivery in chitosan-based 3D constructs to enhance bone regeneration in a rat mandibular defect model. Tissue Eng A. 2014;20(15-16):2169-2179. https://doi.org/10.1089/ten.tea.2013.0523

49. Chen Z, Kang L, Meng QY, et al. Degradability of injectable calcium sulfate/mineralized collagen-based bone repair material and its effect on bone tissue regeneration. Mater Sci Eng C. 2014;45:94-102. https://doi.org/10.1016/j.msec.2014.08.060

50. Konopnicki S, Sharaf B, Resnick C, et al. Tissue-engineered bone with 3-dimensionally printed β-tricalcium phosphate and poly-caprolactone scaffolds and early implantation: an in vivo pilot study in a porcine mandible model. J Oral Maxillofac Surg. 2015;73(5):1016.e1-1016.e11. https://doi.org/10.1016/j.joms.2015.01.021

51. Chanchevattik S, Teneman M, Feinberg SE, et al. Segmental mandibular bone reconstruction with a carbonate-substituted hydroxyapatite-coated modular endoprosthetic poly(lactic-co-caprolactone) scaffold in Macaca fascicularis. J Biomed Mater Res B: Appl Biomater. 2014;102(5):962-976. https://doi.org/10.1002/jbm.b.33077

52. Kawai T, Suzuk K, Matsui K, Tanuma Y, Takahashi T, Kamakura S. Octacalcium phosphate collagen composite facilitates bone regeneration of large mandibular bone defect in humans. J Tissue Eng Regen Med. 2017;11(5):1641-1647. https://doi.org/10.1002/term.2110

53. Heo D, Castro N, Lee S-J, Noh H, Zhu W, Zhang LG. Enhanced bone tissue regeneration using 3D printed microstructure incorporated with hybrid nano hydrogel. Physiol Behav. 2017;9(16):5055-5062. https://doi.org/10.1016/j.physbeh.2017.03.040

54. Wang X, Wu X, Xing H, et al. Porous nano hydroxyapatite/collagen scaffolds loading insulin PLGA particles for restoration of critical size bone defect. ACS Appl Mater Interfaces. 2017;9(13):11380-11391. https://doi.org/10.1021/acsami.6b13566
56. Song Y, Zhang C, Wang P, et al. Engineering bone regeneration with novel cell-laden hydrogel microfiber-injectable calcium phosphate scaffold. Mater Sci Eng C. 2017;75:895-905. https://doi.org/10.1016/j.msec.2017.02.158

57. Maglione M, Spano S, Ruaro ME, et al. In vivo evaluation of chitosan-glycerol gel scaffolds seeded with stem cells for full-thickness mandibular bone regeneration. J Oral Sci. 2017;59(2):225-232. https://doi.org/10.20334/josnsud.16-0235

58. Rahman MS, Rana MM, Spitzhorn L-S, et al. Fabrication of biocompatible porous scaffolds based on hydroxyapatite/collagen/chitosan composite for restoration of defected maxillofacial mandible bone. Prog Biomater. 2019;8:137-154. https://doi.org/10.1007/s40204-019-0113-x

59. Lopez CD, Díaz-Siso JR, Witek L, et al. 3D printed bioactive ceramic scaffold osseoconduction across critical-sized mandibular defects. Physiol Behav. 2017;176:139-148. https://doi.org/10.1016/j.physbeh.2017.03.040

60. Roskies MG, Fang D, Abdallah MN, et al. Three-dimensionally printed polyetherketoneketone scaffolds with mesenchymal stem cells for the reconstruction of critical-sized mandibular defects. Laryngoscope. 2017;127(11):E392-E398. https://doi.org/10.1002/ 1ary.26781

61. Wang Y, Papagerakis S, Faulk D, et al. Extracellular matrix membrane induces cementoblastic/osteoegenic properties of human periodontal ligament stem cells. Front Physiol. 2018;9:1-11. https://doi.org/10.3389/fphys.2018.00942

62. Elkhateeb L, Zohdy A, Atalla SS, Moussa MH, Hamam GG, Zahra FAEA. Comparative study on acellular dermal graft versus propylene mesh both either loaded or unloaded with BM-MSCs in healing of skull bone defect in rats: histological and immunohistochemical study. Int J Stem Cells. 2018;11(2):216-226. https://doi. org/10.15283/ijsc18019

63. Rajan A, Eubanks E, Edwards S, et al. Optimized cell survival and seeding efficiency for craniofacial tissue engineering using clinical stem cell therapy. Stem Cells Transl Med. 2014;3(12):1495-1503. https://doi.org/10.5966/scm.2014.0039

64. Redondo LM, Garcia V, Peral B, et al. Repair of maxillary cystic bone defects with mesenchymal stem cells seeded on a cross-linked serum scaffold. J Cranio-Maxillofac Surg. 2018;46(2):222-229. https://doi.org/10.1016/j.joms.2017.11.004

65. Ma B, Han J, Zhang S, et al. Hydroxyapatite nanobelot/polyactic acid Janus membrane with osteoinduction/barrier dual functions for precise bone repair. Acta Biomater. 2018;71:108-117. https://doi.org/10.1016/j.actbio.2018.02.033

66. Shafieian R, Matin MM, Rahpeyma A, et al. Effects of human adipose-derived stem cells and platelet-rich plasma on healing response of canine alveolar surgical bone defects. Arch Oral Biol. 2017;5(6):404-416. https://doi.org/10.22038/abs.2017.23121.1612

67. Hu LW, Wang X, Jiang QX, Xu LQ, Pan HY. In vivo and in vitro study of osteogenic potency of endotelin-1 on bone marrow-derived mesenchymal stem cells. Exp Cell Res. 2017;357(1):25-32. https://doi.org/10.1016/j.yexcr.2017.04.018

68. Du B, Gao Y, Deng Y, et al. Local delivery of rhVEGF165 through biocoated nHA/coral grafts in critical-sized dog mandible defects: a histological study at the early stages of bone healing. Int J Clin Exp Med. 2015;8(4):4940-4953. http://www.embase.com/search/results?subpanel=viewrecord&from=export&id=L604831394%5Cnhttp://limo.libis.be/resolver?&sid=EMBASE&issn=194059016&doi=&title=Local+delivery+of+rhVEGF%C3%81+C+through+biocoated+nHA/coral+graft+in+crit 

69. Khojastehe D, Dashki SG, Dehghan MM, Behnia H, Abbasiap N, Morad G. The osteoregenerative effects of platelet-derived growth factor BB coimplanted with mesenchymal stem cells, loaded on freeze-dried mineral bone block: a pilot study in dog mandible. J Biomed Mater Res B: Appl Biomater. 2014;102(8):1771-1778. https://doi.org/10.1002/jbm.b.33156

70. Khojastehe A, Fahimipour F, Jafarian M, et al. Bone engineering in dog mandible: coculturing mesenchymal stem cells with endothelial progenitor cells in a composite scaffold containing vascular endothelial growth factor. J Biomed Mater Res B: Appl Biomater. 2017;105(7): 1767-1777. https://doi.org/10.1002/jbm.b.33707

71. Suliman S, Xing Z, Wu X, et al. Release and bioactivity of bone morphogenetic protein-2 are affected by scaffold binding techniques in vitro and in vivo. J Control Release. 2015;197:148-157. https://doi. org/10.1016/j.jconrel.2014.11.003

72. Peng Z, Liu J, Shen C, et al. Biotin-avidin mediates the binding of adipo-derived stem cells to a porous β-tricalcium phosphate scaffold: mandibular regeneration. Exp Ther Med. 2016;11(3):737-746. https://doi.org/10.3892/etm.2015.2961

73. Tong S, Amand C, Kieffer A, Kyaw MH. Trends in healthcare utilization and costs associated with acute otitis media in the United States during 2008-2014. BMC Health Serv Res. 2018;18(1):1-10. https://doi.org/10.1186/s12913-018-3139-1

74. Tee BC, Desai KGH, Kennedy KS, et al. Reconstructing jaw defects with MSCs and PLGA-encapsulated growth factors. Am J Transl Res. 2016;8(6):2693-2704.

75. Çakır-Özkan N, Egri S, Bekar E, Altunkaynak BZ, Kabak YB, Kivrak EG. The use of sequential VEGF- and BMP2-releasing biodegradable scaffolds in rabbit mandibular defects. J Oral Maxillofac Surg. 2017;75(1):221.e1-221.e14. https://doi.org/10.1016/j.joms.2016.08.020

76. Du B, Liu W, Deng Y, et al. Angiogenesis and bone regeneration of porous nano-hydroxyapatite/coralline blocks coated with rhVEGF165 in critical-size alveolar bone defects in vivo. Int J Nanomed. 2015;10: 2555-2565. https://doi.org/10.2147/IJN.S78331

77. Tong S, Xu DP, Liu ZM, Du Y, Wang XK. Synthesis of and in vitro and in vivo evaluation of a novel TGF-1-SF-CS three-dimensional scaffold for bone tissue engineering. Int J Mol Sci. 2016;18(3):257-380. https://doi.org/10.3390/ijms.16.2651

78. Xie Y, Su Y, Min S, et al. Erratum to “Collagen sponge functionalized with chimeric anti-BMP-2 monoclonal antibody mediates repair of critical-size mandibular continuity defects in a nonhuman primate model”. Biomembranes Int. 2017:2017/368977

79. Deng N, Sun J, Li Y, et al. Experimental study of rhBMP-2 chitosan nano-sustained-release carrier-loaded PLGA/nHA scaffolds to construct mandibular tissue-engineered bone. Arch Oral Biol. 2019;102:16-25. https://doi.org/10.1016/j.archoralbio.2019.03.023

80. Tatar AM, Wong ME, Mikos AG. In vivo bone bioreactors for mandibular reconstruction. J Dent Res. 2014;93(12):1196-1202. https://doi.org/10.1177/0022034514514976

81. Naujokat H, Açil Y, Gülsen A, Birkenfeld F, Wittfang J. Man as a living bioreactor: long-term histological aspects of a mandibular replacement engineered in the patient’s own body. Int J Oral Maxillofac Surg. 2018;47(11):1481-1487. https://doi.org/10.1016/j.ijo.2018.05.006

82. Wittfang J, Rohnen M, Egberts J, et al. Man as a living bioreactor: prefabrication of a custom vascularized bone graft in the gastrocrocid omentum. Tissue Eng C: Methods. 2016;22(8):740-746.

83. Tatar AM, Kretlow JD, Spicer PP, et al. Autologously generated tissue-engineered bone flaps for reconstruction of large mandibular defects in an ovine model. Tissue Eng A. 2015;21(9-10):1520-1528. https://doi.org/10.1089/ten.tea.2014.0426

84. Naujokat H, Açil Y, Harder S, Lipp M, Böhmsen F, Wittfang J. Osseointegration of dental implants in ectopic engineered bone in three different scaffold materials. Int J Oral Maxillofac Surg. 2020; 49(1):135-142. https://doi.org/10.1016/j.iomms.2019.04.005

85. Brookes S, Voytik-Harbin S, Zhang H, Zhang L, Halum S. Motor endplate-expressing cartilage-muscle implants for reconstruction of
a denervated hemilarynx. Laryngoscope. 2019;129(6):1293-1300. https://doi.org/10.1002/lary.27575

86. Fukuhori M, Chitose SI, Sato K, et al. Regeneration of vocal fold mucosa using tissue-engineered structures with oral mucosal cells. PLoS One. 2016;11(1):1-15. https://doi.org/10.1371/journal.pone.0146151

87. Herrmann P, Ansari T, Southgate A, et al. In vivo implantation of a tissue engineered stem cell seeded hemi-laryngeal replacement maintains airway, phonation, and swallowing in pigs. J Tissue Eng Regen Med. 2019;13(11):1943-1954. https://doi.org/10.1002/term.2596

88. Chiang T, Pepper V, Best C, et al. Clinical translation of tissue engineered trachea grafts. Ann Otol Rhinol Laryngol. 2016;125(11):873-885. https://doi.org/10.1177/0003489416656646

89. Macchiarini P, Jungeluth P, Go T, et al. Clinical translation of a tissue-engineered airway. Lancet. 2008;372(9655):2026-2030. https://doi.org/10.1016/S0140-6736(08)61598-6

90. Gonfianti A, Jaus M, Barale D, et al. The first tissue-engineered airway transplantation: 5-year follow-up results. Lancet. 2014;383(9913):238-244. https://doi.org/10.1016/S0140-6736(13)62033-4

91. Maughan EF, Butler CR, Crowley C, et al. A comparison of tracheal scaffold strategies for pediatric transplantation in a rabbit model. Laryngoscope. 2017;127(12):E449-E457. https://doi.org/10.1002/lary.26611

92. Zhao L, Sundaram S, Le AV, et al. Engineered tissue-stent biocomposites as tracheal replacements. Tissue Eng A. 2016;22(17-18):1086-1097. https://doi.org/10.1089/teng.2016.0132

93. Dharadhikari S, Best CA, King N, et al. Mouse model of tracheal replacement with electrospun nanofiber scaffolds. Ann Otol Rhinol Laryngol. 2019;128(5):391-400. https://doi.org/10.1177/0003489419826134

94. Best CA, Pepper VK, Ohst D, et al. Designing a tissue-engineered tracheal scaffold for preclinical evaluation. Int J Pediatr Otorhinolaryngol. 2018;104:145-150. https://doi.org/10.1016/j.ijpfo.2017.10.036

95. Sharp JF, Denholm S. Routine X-rays in nasal trauma: the influence of scaffolding properties for chondrogenic differentiation of adipose-derived mesenchymal stem cells in nasal reconstruction. JAMA Facial Plast Surg. 2017;19(2):108-114. https://doi.org/10.1001/jmfa.2016.1200

96. Watson D, Reuther MS, Wong VW, Sah RL, Masuda K, Briggs KK. Effect of hyaluronidase on tissue engineered human septal cartilage. Laryngoscope. 2016;126(9):1984-1989. https://doi.org/10.1002/lary.25884

97. Batioglu-Karaaltin A, Karaaltin MV, Ovali E, et al. Vascularization of xenogeneic collagen scaffold in nasal cartilage repair. Tissue Eng A. 2014;20(11-12):1668-1678. https://doi.org/10.1089/ten.tea.2013.0365

98. Reighard CL, Hollister SJ, Zopf DA. Auricular reconstruction from rib to 3D printing. J 3D Print Med. 2018;2(1):35-41. https://doi.org/10.2217/3dp-2017-0017

99. Lin CH, Yang IC, Tsai CH, Fang HW, Ma H. Auricular tissue engineering using osteogenic differentiation of adipose stem cells with small intestine submucosa. Plast Reconstr Surg. 2017;140(2):297-305. https://doi.org/10.1097/PRS.0000000000003522

100. Cohen BP, Bernstein JL, Morrison KA, Spector JA, Bonassar LJ. Tissue engineering the human auricle by auricular chondrocyte-mesenchymal stem cell co-implantation. PLoS One. 2018;13(10):1-19. https://doi.org/10.1371/journal.pone.0202356

101. Nakao H, Jacquet RD, Shasti M, Isogai N, Murthy AS, Landis WJ. Microencapsulated rabbit adipose stem cells initiate tissue regeneration in a rabbit ear defect model. J Tissue Eng Regen Med. 2018;12(7):1742-1753. https://doi.org/10.1002/term.2702
118. Duïsit J, Amiel H, Wüthrich T, et al. Perfusion-decellularization of human ear grafts enables ECM-based scaffolds for auricular vascularized composite tissue engineering. Acta Biomater. 2018;73:339-354. https://doi.org/10.1016/j.actbio.2018.04.009

119. Bernstein JL, Cohen BP, Lin A, Harper A, Bonasser LJ, Spector JA. Tissue engineering auricular cartilage using live passage human auricular cartilage. Ann Plast Surg. 2018;80(4):S168-S173. https://doi.org/10.1093/ajps/jpy167.2017.03.040

120. Bos EJ, Blumekeers M, Helder M, et al. Structural and mechanical comparison of human ear, alar, and septal cartilage. Plast Reconstr Surg - Glob Open. 2018;6(1):e-1. https://doi.org/10.1097/GOX.0000000000001610

121. He A, Xia H, Xiao K, et al. Cell yield, chondrogenic potential, and regeneration cartilage type of chondrocytes derived from ear, nasoseptal, and costal cartilage. J Tissue Eng Regen Med. 2016;12(4):1123-1132. https://doi.org/10.1002/term.2613

122. Lee JS, Kim BS, Seo D, Park JH, Cho DW. Three-dimensional cell printing of large-volume tissues: application to ear regeneration. Tissue Eng C. Methods. 2017;23(3):136-145. https://doi.org/10.1089/ten.tec.2016.0362

123. Karimi H, Emami SA, OIad-Gubad MK. Bone marrow stem cells and novel plasma-derived albumin scaffolds. Acta Otolaryngol. 2017;137(4):432-441. https://doi.org/10.1080/00016489.2016.1257151

124. Gu Y, Kang N, Dong P, et al. Chondrocytes from congenital microtia possess an inferior capacity for in vivo cartilage regeneration to healthy ear chondrocytes. J Tissue Eng Regen Med. 2018;12(3):e1737-e1746. https://doi.org/10.1002/term.2359

125. Robla Costales D, Junquera L, García Pérez E, Gómez Llamés S, Álvarez-Viejo M, Meana-Infiesta /C19. Ectopic bone formation during auricular cartilage alternative. J Biomed Mater Res B: Appl Biomater. 2017;105(5):1016-1028. https://doi.org/10.1002/jbmr.33639

126. Kagimoto S, Takebe T, Kobayashi S, et al. Chondrocyte differentiation for auricular cartilage reconstruction with nondegradable porous polyurethane scaffolds as a potential auricular cartilage alternative. J Biomed Mater Res B: Appl Biomater. 2017;105(5):1477-1485. https://doi.org/10.1002/jbmr.33529

127. Wang Z, Qin H, Feng Z, Zhao Y. Platelet-rich plasma gel composited with alginate bioink for cartilage tissue engineering applications. Biomacromolecules. 2015;16(5):1489-1496. https://doi.org/10.1021/acs.biomac.5b00188

128. Martínez Ávila H, Feldmann EM, Pleumeekers MM, et al. Novel bilayer bacterial nanocellulose scaffold supports neocartilage formation invitro and invivo. Biomaterials. 2015;44:122-133. https://doi.org/10.1016/j.biomaterials.2014.12.025

129. Zopf DA, Mitsak AG, Flanagan CL, Wheeler M, Green GE, Hollister SJ. Computer-aided designed, 3-dimensionally printed porous tissue bioscaffolds for craniofacial soft tissue reconstruction. Otolaryngol Head Neck Surg. 2015;152(1):57-62. https://doi.org/10.1177/0194599814552065.Computer-Aided

130. O'Sullivan NA, Kobayashi S, Ranka MP, et al. Adhesion and integration of tissue engineered cartilage to porous polyethylene for composite ear reconstruction. J Biomed Mater Res B: Appl Biomater. 2015;103(5):983-991. https://doi.org/10.1002/jbm.b.33269

131. Cheng Y, Cheng P, Xue F, et al. Repair of ear cartilage defects with adipose-derived stem cells and acellular cartilaginous matrix in rabbits. Genet Mol Res. 2014;13(2):4599-4606. https://doi.org/10.4238/2014.June.18.2

132. Cheng Y, Cheng P, Xue F, et al. Repair of ear cartilage defects with allogeneic bone marrow mesenchymal stem cells in rabbits. Cell Biochem Biophys. 2014;70(2):1137-1143. https://doi.org/10.1007/s12013-014-0033-2

133. Xia H, Zhao D, Zhu H, et al. Lyophilized scaffolds fabricated from 3D-printed photocurable natural hydrogel for cartilage regeneration. ACS Appl Mater Interfaces. 2018;10(37):31704-31715. https://doi.org/10.1021/acsami.8b09026

134. Naik A, Griffin MF, Szarko M, Butler PE. Optimizing the decellularization process of human maxillofacial muscles for facial reconstruction using a detergent-only approach. J Tissue Eng Regen Med. 2019;13(9):1571-1580. https://doi.org/10.1002/term.2910

135. Bayar GR, Kuo S, Marcelo CL, Feinberg SE. In vitro development of a mucocutaneous junction for lip reconstruction. J Oral Maxillofac Surg. 2016;74(11):2317-2326. https://doi.org/10.1016/j.joms.2016.04.002

136. Zhao C, Qazvini NT, Sadati M, et al. A pH-triggered, self-assembled, and bioprintable hybrid hydrogel scaffold for mesenchymal stem cell based bone tissue engineering. ACS Appl Mater Interfaces. 2019;11(9):8749-8762. https://doi.org/10.1021/acsami.8b19094
149. Lin Y, Umebayashi M, Abdallah MN, et al. Combination of polyetherketonelactone scaffold and human mesenchymal stem cells from temporomandibular joint synovial fluid enhances bone regeneration. Sci Rep. 2019;9(1):1-13. https://doi.org/10.1038/s41598-018-36778-2

150. Yu D, Zhao X, Cheng JZ, Wang D, Zhang HH, Han GH. Downregulated microRNA-488 enhances odontoblast differentiation of human dental pulp stem cells via activation of the p38 MAPK signaling pathway. J Cell Physiol. 2019;234(2):1442-1451. https://doi.org/10.1002/jcp.26950

151. Kim YJ, Park SG, Shin B, et al. Osteogenesis for postoperative temporal bone defects using human ear adipose-derived stromal cells and tissue engineering: an animal model study. J Biomed Mater Res A. 2017;105(12):3493-3501. https://doi.org/10.1002/jbma.36194

152. Dan Y, Liu O, Liu Y, et al. Development of novel biocomposite scaffold of chitosan-gelatin/nanohydroxyapatite for potential bone tissue engineering applications. Nanoscale Res Lett. 2016;11(1):1-6. https://doi.org/10.1186/s11671-016-1669-1

153. Jang CH, Cho YB, Choi CH, et al. Effect of umbilical cord serum coated 3D PCL/alginate scaffold for mastoid obliteration. Int J Pediatr Otology. 2014;78(7):1061-1065. https://doi.org/10.1016/j.ijo.2014.04.004

154. Bardsley K, Kwarciak A, Freeman C, Brook I, Hatton P, Crawford A. Bone tissue engineering using engineered bone grafts for alveolar cleft osteoplasty in a rodent model. J Cranio-Maxillofac Surg. 2015;43(8):985-994. https://doi.org/10.1016/j.jcmax.2016.04.012

155. Adamzyk C, Kachel P, Hoss M, et al. Bone tissue engineering using engineered bone grafts for mandibular defects. J Biomed Mater Res A. 2016;10414

156. Hixon KR, Melvin AM, Lin AY, Hall AF, Sell SA. Biocompatibility studies of nanoengineered polycaprolactone and nanohydroxyapatite scaffold for craniofacial bone regeneration. J Craniofac Surg. 2019;30(1):265-269. https://doi.org/10.1097/SCS.0000000000004857

157. Han HH, Shim JH, Lee H, et al. Reconstruction of complex maxillary defects using patient-specific 3D-printed biodegradable scaffolds.
preliminary report. Artif Organs. 2014;38(6):E95-E105. https://doi.org/10.1111/aor.12310

213. Park JH, Yoon JK, Lee JB, et al. Experimental tracheal replacement using 3-dimensional bioprinted artificial trachea with autologous epithelial cells and chondrocytes. Sci Rep. 2019;9(1):1-11. https://doi.org/10.1038/s41598-019-38565-z

214. Butler CR, Hynds RE, Crowley C, et al. Vacuum-assisted decellularization: an accelerated protocol to generate tissue-engineered human tracheal scaffolds. Biomaterials. 2017;124:95-105. https://doi.org/10.1016/j.biomaterials.2017.02.001

215. Pleumeekers MM, Nimeskern L, Koevoet WLM, Karperien M, Stok KS, Van Osch GJVM. Cartilage regeneration in the head and neck area: combination of ear or nasal chondrocytes and mesenchymal stem cells improves cartilage production. Plast Reconstr Surg. 2015;136(6):762e-774e. https://doi.org/10.1097/PRS.0000000000001812

216. Sueyoshi S, Chitose SI, Sato K, Fukahori M, Kurita T, Umeno H. Stable tracheal regeneration using organotypically cultured tissue composed of autologous chondrocytes and epithelial cells in beagles. Ann Otol Rhinol Laryngol. 2019;128(7):585-594. https://doi.org/10.1177/0003484919343313

217. Al-Ayoubi AM, Rehmani SS, Sinclair CF, Lebovics RS, Bhora FY. Cartilage regeneration in the head and neck area: combination of ear or nasal chondrocytes and mesenchymal stem cells improves cartilage production. Plast Reconstr Surg. 2015;136(6):762e-774e. https://doi.org/10.1097/PRS.0000000000001812

218. Ahn CB, Son KH, Yu YS, Kim TH, Lee JI, Lee JW. Development of a flexible 3D printed scaffold with a cell-adhesive surface for artificial trachea. Biomed Mater. 2019;14(5):1-22.

219. Guimaraes AB, Correia AT, Alves BP, et al. Evaluation of a physical-chemical protocol for porcine tracheal decellularization. Transplant Proc. 2019;51(5):1611-1613. https://doi.org/10.1016/j.transproceed.2019.01.042

220. Umeda S, Nakayama Y, Umeda S, Takama Y, Terazawa T, Okuyama H. Tracheal replacement using an in-body tissue-engineered collagenous tube BIOTUBE with a biodegradable stent in a beagle model: a preliminary report on a new technique. Eur J Pediatr Surg. 2019;29(1):90-96. https://doi.org/10.1055/s-0038-1673709

221. Zhang Y, Xu Y, Liu Y, et al. Porous decellularized tracheal scaffold prepared by a laser micropore technique. J Mech Behav Biomed Mater. 2019;90:96-103. https://doi.org/10.1016/j.jmbbm.2018.10.006

222. Chang CS, Yang CY, Hsiao HY, et al. Cultivation of auricular chondrocytes in poly(ethylene glycol)/poly(ε-caprolactone) hydrogel for tracheal cartilage tissue engineering in a rabbit model. Eur Cells Mater. 2018;35:350-364. https://doi.org/10.22203/ECM.v035a24

223. Bae SW, Lee KW, Park JH, et al. 3D bioprinted artificial trachea with epithelial cells and chondrogenic-differentiated bone marrow-derived mesenchymal stem cells. Int J Mol Sci. 2018;19(6):1-14. https://doi.org/10.3390/ijms19061624

224. Gilevich IV, Sotinchenko AS, Karal-ogly DD, et al. In vivo experimental study of biological compatibility of tissue engineered tracheal construct in laboratory primates. Bull Exp Biol Med. 2018;164(6):770-774. https://doi.org/10.1007/s10517-018-4077-y

225. Jing H, Gao B, Gao M, et al. Restoring tracheal defects in a rabbit model with tissue-engineered cartilage by TGF-β3-encapsulating electrospun poly(l-lactic acid-co-ε-caprolactone)/collagen scaffolds. Artif Cells Nanomed Biotechnol. 2018;46(suppl 1):985-995. https://doi.org/10.1080/21691401.2018.1439844

226. Lu T, Huang Y, Qiao Y, Zhang Y, Liu Y. Evaluation of changes in cartilage viability in detergent-treated tracheal grafts for immunosuppressant-free allotransplantation in dogs. Eur J Cardio-Thorac Surg. 2018;53(3):672-679. https://doi.org/10.1093/ejcts/ezs317

227. Ghobani F, Moradi L, Shadmehr MB, Bonakdar S, Drodinia A, Safshekan F. In-vivo characterization of a 3D hybrid scaffold based on PCL/decellularized aorta for tracheal tissue engineering. Mater Sci Eng C. 2017;71:74-83. https://doi.org/10.1016/j.msec.2017.04.150

228. Han Y, Wang Y, Li J, et al. Biomechanical properties and cellular bio-compatibility of 3D printed tracheal graft. Bioprocess Biosyst Eng. 2017;40(12):1813-1823. https://doi.org/10.1007/s00449-017-1835-6

229. Ohno M, Fujimoto Y, Hsu HC, et al. Airway reconstruction using decellularized tracheal allografts in a porcine model. Pediatr Surg Int. 2017;33(10):1065-1071. https://doi.org/10.1007/s00383-017-4138-8

230. Gao M, Zhang H, Dong W, et al. Tissue-engineered trachea from a 3D-printed scaffold enhances whole-segment tracheal repair. Sci Rep. 2017;7(1):1-12. https://doi.org/10.1038/s41598-017-05518-3

231. Taniguchi D, Matsumoto K, Tsuchiya T, et al. Scaffold-free trachea regeneration by tissue engineering with bio-3D printing. Interact.
277. Jang YS, Jang CH, Cho YB, Kim M, Kim GH. Tracheal regeneration using polycaprolactone/collagen-nanofiber coated with umbilical cord serum after partial resection. *Int J Pediatr Otorhinolaryngol*. 2014;78(12):2237-2243. https://doi.org/10.1016/j.ijporl.2014.10.022

278. Steinke M, Dally I, Friedel G, Walles H, Walles T. Host-integration of a tissue-engineered airway patch: two-year follow-up in a single patient. *Tissue Eng A*. 2015;21(3-4):573-579. https://doi.org/10.1089/ten.tea.2014.0200

279. Shin YS, Choi JW, Park JK, et al. Tissue-engineered tracheal reconstruction using mesenchymal stem cells seeded on a porcine cartilage powder scaffold. *Ann Biomed Eng*. 2015;43(4):1003-1013. https://doi.org/10.1007/s10439-014-1126-1

280. Evaristo TC, da Cruz Alves FCM, Moroz A, et al. Light-emitting diode effects on combined decellularization of trachea. A novel approach to obtain biological scaffolds. *Acta Cir Bras*. 2014;29(8):485-492. https://doi.org/10.1590/S0102-86502014000800002

281. Jungebluth P, Haag JC, Sjöqvist S, et al. Tracheal tissue engineering mice using the body as a natural bioreactor. *Biomed Mater Res A*. 2014;1089/ten.tea.2014.0089

282. Jones MC, Ruegggeber FA, Cunningham AJ, et al. Biomechanical changes from long-term freezer storage and cellular reduction of tracheal scaffolds. *Laryngoscope*. 2015;125(1):E16-E22. https://doi.org/10.1002/lary.24853

283. Kajbafzadeh AM, Sabetkish S, Sabetkish N, et al. In-vivo trachea regeneration: fabrication of a tissue-engineered trachea in nude mice using the body as a natural bioreactor. *Surg Today*. 2015;45(8):1040-1048. https://doi.org/10.1007/s00595-014-0993-2

284. Sun F, Pan S, Shi HC, et al. Structural integrity, immunogenicity and biomechanical evaluation of rabbit decellularized tracheal matrix. *J Biomed Mater Res A*. 2015;103(4):1509-1519. https://doi.org/10.1002/jbm.a.35273

285. Lee JH, Park HS, Oh SH, et al. Triple-layered polyurethane prosthesis with wrinkles for repairing partial tracheal defects. *Laryngoscope*. 2014;124(12):2757-2763. https://doi.org/10.1002/lary.24809

286. Kuten JC, McGovern D, Hobson CM, et al. Decellularized tracheal extracellular matrix supports epithelial migration, differentiation, and function. *Tissue Eng A*. 2015;21(1-2):75-84. https://doi.org/10.1089/ten.tea.2014.0089

287. Hamaji M, Kojima F, Koyasu S, et al. Development of a composite and vascularized tracheal scaffold in the omentum for in situ tissue engineering: a canine model. *Interact Cardiovasc Thorac Surg*. 2014;19(3):357-362. https://doi.org/10.1093/icvts/ivu177

288. Baigueria S, Del Gaudio C, Kuevda E, Gonfiotti A, Bianco A, Macchiarini P. Dynamic decellularization and cross-linking of rat tracheal matrix. *Ann Biomed Eng*. 2014;43(24):6344-6350. https://doi.org/10.1007/s10439-014-1126-1

289. Haykal S, Salna M, Zhou Y, et al. Double-chamber rotating bioreactor for dynamic perfusion cell seeding of large-segment tracheal allografts: comparison to conventional static methods. *Tissue Eng C: Methods*. 2014;20(8):681-692. https://doi.org/10.1089/ten.tec.2013.0627

290. Oldenburg MS, Ekborn DC, San Marina S, et al. Preliminary results of tissue-engineered injection laryngoplasty material in a rabbit model. *Laryngoscope*. 2018;128(1):160-167. https://doi.org/10.1002/lary.26849

291. Imaiizu M, Li-Jessen NYK, Sato Y, Yang DT, Thibeault SL. Retention of human-induced pluripotent stem cells (hiPS) with injectable HA hydrogels for vocal fold engineering. *Ann Otol Rhinol Laryngol*. 2017;126(4):304-314. https://doi.org/10.1177/0003489417691296

292. Huang D, Wang R, Yang S. Cogels of hyaluronic acid and acellular matrix for cultivation of adipose-derived stem cells: potential application for vocal fold tissue engineering. *Biomed Res Int*. 2016;2016(2):1-10. https://doi.org/10.1155/2016/6584054

293. Wrona EA, Peng R, Born H, Amin MR, Branski RC, Freytes DO. Derivation and characterization of porcine vocal fold extracellular matrix scaffold. *Laryngoscope*. 2016;126(4):928-935. https://doi.org/10.1002/lary.25640

294. Hong HJ, Chang JW, Park JK, et al. Tracheal reconstruction using chondrocytes seeded on a poly(L-lactic-co-glycolic acid)-fibrin/hyaluronic. *J Biomed Mater Res A*. 2014;102(11):4142-4150. https://doi.org/10.1002/jbm.a.35091

295. Palencia L, Das A, Palecek SP, Thibeault SL, Leydon C. Epidermal growth factor mediated healing in stem cell-derived vocal fold mucosa. *J Surg Res*. 2015;197(1):32-38. https://doi.org/10.1016/j.jsrs.2015.02.066

296. Sun A, Meng Q, Li W, Liu S, Chen W. Construction of tissue-engineered laryngeal cartilage with a hollow, semi-flared shape using poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) as a scaffold. *Exp Ther Med*. 2015;9(4):1482-1488. https://doi.org/10.3892/etm.2015.2262

297. Gaston J, Bartlett R, Klemuk S, Thibeault S. Formulation and characterization of a porous, elastomeric biomaterial for vocal fold tissue engineering research. *Ann Otol Rhinol Laryngol*. 2014;123(12):866-874. https://doi.org/10.1080/jid.2014.371

298. Mau T, Du M, Xu C. A rabbit vocal fold laser scarring model for testing lamina propria tissue-engineering therapies. *Laryngoscope*. 2014;124(10):2321-2326. https://doi.org/10.1002/lary.24707

299. Ansari T, Lange P, Southgate A, et al. Stem cell-based tissue-engineered laryngeal replacement. *Tissue Eng Regen Med*. 2017;6:677-687.

300. Wismayer K, Mehrban N, Bowen J, Birchall M. Improving cellular migration in tissue-engineered laryngeal scaffolds. *J Laryngol Otol*. 2019;133(2):135-148. https://doi.org/10.1017/S0022215119000082

301. Mehrban N, Bowen J, Tait A, et al. Silsesquioxane polymer as a potential scaffold for laryngeal reconstruction. *Mater Sci Eng C*. 2018;92:565-574. https://doi.org/10.1016/j.msec.2018.07.003

302. Zhang H, Voytik-Harbin S, Brookes S, et al. Use of autologous adipose-derived mesenchymal stem cells for creation of laryngeal cartilage. *Physiol Behav*. 2018;128(4):E123-E129. https://doi.org/10.1016/j.physbeh.2017.03.040

303. Brookes S, Voytik-Harbin S, Zhang H, Halum S. 3-Dimensional (3D) tissue-engineered skeletal muscle for laryngeal reconstruction. *Laryngoscope*. 2018;128(3):603-609. https://doi.org/10.1177/1214909818.002567

304. Jacobs IN, Redden RA, Goldberg R, et al. Pediatric laryngotracheal reconstruction with tissue-engineered cartilage in a rabbit model. *Laryngoscope*. 2016;126(5):521-531. https://doi.org/10.1002/lary.25676

305. Tse JR, Long JL. Microstructure characterization of a decellularized vocal fold scaffold for laryngeal tissue engineering. *Laryngoscope*. 2014;124(8):E326-E331. https://doi.org/10.1002/lary.24605

306. Kitamura M, Hirano S, Kanemaru S, et al. Glottic regeneration with a tissue-engineered vocal fold scaffold for laryngeal tissue engineering. *Otolaryngology Investigative Otolaryngology*. 2020;5:630-648. https://doi.org/10.1002/loit.1.2416