Regenerative medicine for end-stage fibrosis and tissue loss in the upper aerodigestive tract: a twenty-first century review

Meta Analysis Services

Abstract

Objective. This review assesses regenerative medicine of the upper aerodigestive tract during the first two decades of the twenty-first century, focusing on end-stage fibrosis and tissue loss in the upper airways, salivary system, oropharynx and tongue.

Method. PubMed, Embase, Google Scholar, Cochrane Library, Medline and clinicaltrials.org were searched from 2000 to 2019. The keywords used were: bioengineering, regenerative medicine, tissue engineering, cell therapy, regenerative surgery, upper aerodigestive tract, pharynx, oropharynx, larynx, trachea, vocal cord, tongue and salivary glands. Original studies were subcategorised by anatomical region. Original human reports were further analysed. Articles on periodontology, ear, nose and maxillofacial disorders, and cancer immunotherapy were excluded.

Results. Of 716 relevant publications, 471 were original studies. There were 18 human studies included, within which 8 reported airway replacements, 5 concerned vocal fold regeneration and 3 concerned salivary gland regeneration. Techniques included cell transplantation, injection of biofactors, bioscaffolding and bioengineered laryngeal structures.

Conclusion. Moderate experimental success was identified in the restoration of upper airway, vocal fold and salivary gland function. This review suggests that a shift in regenerative medicine research focus is required toward pathology with a higher disease burden

Introduction

Regenerative medicine describes research and clinical applications that aim to repair, replace or regenerate cells, tissue and organs via cell therapies, gene editing or tissue engineering. Over the past two decades, research activity in regenerative medicine has grown exponentially across all medical disciplines. Despite well-publicised examples of functional restoration across a wide range of organs, few regenerative treatments have gained licence for routine clinical use in the USA and Europe. Many are with□drawn because of poor efficacy, safety concerns or financial insolvency. Regenerative medicine aims to harness intrinsic cellular mechanisms for tissue or organ regeneration. This variably involves tissue engineering, cell biology, gene therapy and stem cell therapy. Alongside other new technologies, regenerative medicine is claimed to follow the Gartner Hype Cycle (Figure 1). Technological triggers in the 1980s and 1990s encouraged optimism, investment and expectations of swift mass-market success. However, limited existing technology and scalability issues prevented a breakthrough to routine clinical use, prompting disillusionment. Currently, regenerative medicine is reck□oned to be in the third phase of the cycle, a plateau of productivity before treatment will reach patients effectively and economically. Therefore, investments in tissue-engineered medicinal products are predicted to grow from $10.8 billion in 2016 to $22.3 billion in 2025.

Regenerative medicine applications for regions of the upper aerodigestive tract (mouth, throat, larynx and upper airways) are growing. These regions permit rapid tissue retrieval for ex-vivo cell expansion. They allow for less invasive tissue delivery into and monitoring of treated areas than in organs within enclosed body cavities. There is clear demand: head and neck cancer is the sixth most common cancer globally, with approximately 630 000 new cases each year. Despite relatively high cure rates and survival, treatment with surgical excision, chemotherapy or radiotherapy results in tissue loss and fibrosis within the upper aerodigestive tract. Severe long-term disability may ensue, including loss of voice and swallow, and repositioning of the airway opening into the neck.

The resultant health burden brings difficulties returning to fulltime employment and low quality of life.17,18 Benign disease may also result in end-stage fibrosis within the upper aerodigestive tract, with the same deleterious effect on function.19,20 Regenerative treatments that address fibrosis or replace diseased tissue therefore present an important anatomical tar□get for regenerative medicine over the next decade.

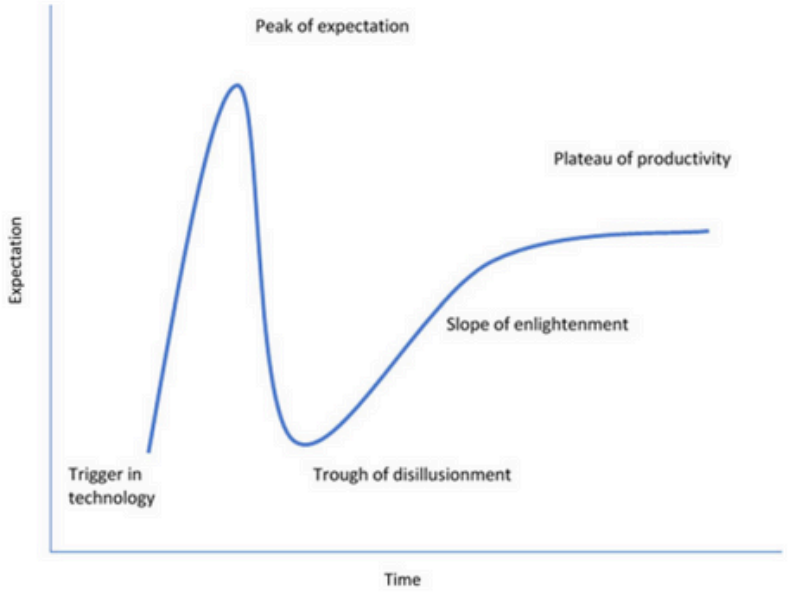

Fig. 1. The Gartner Hype Cycle represents the maturity of emerging technologies. Regenerative medicine is believed to follow this pattern with initial high expectations giving way to disillusionment followed by a slower phase of progress and a plateau of productivity.

This review examines the past twenty years, attempting to identify current focuses in the field and progress toward clinical application and improved patient outcomes. It focuses on upper aerodigestive tract disease affected by end-stage fibrosis and tissue loss because of cancer, surgery, radiation, trauma, age or congenital defects, namely in the throat, salivary system, tongue, larynx and upper airway. Previous studies have demonstrated the complexity of the task involved.21 Some areas of success for regenerative medicine, with established protocols and guidelines, are mentioned

Materials and methods

A systematic literature review on the use of regenerative medicine targeted to the upper aerodigestive tract regions involved in airway, swallow and voice was conducted. PubMed, Embase, Google Scholar, Cochrane Library, Ovid Embase, Ovid Medline databases and clinicaltrials.org were searched between 1 January 2000 and 16 July 2019 for English-language articles. The following keywords were used: bioengineering, regenerative medicine, tissue engineering, cell therapy, regenerative surgery and upper aerodigestive tract, pharynx, oropharynx, larynx, trachea, vocal cord, tongue, and salivary glands. Articles concerning nerve regeneration, periodontology, ear, nose and maxillofacial disorders were excluded. Publications on immunotherapy for cancer were excluded as an exclusive field beyond the scope of this article. Notes, letters and editor□ials with no additional contribution were excluded.

Overall publication data were gathered, and original human studies on regenerative medicine for fibrosis or tissue loss in the trachea, larynx, salivary glands and tongue or pharynx were ana□lysed further. Reference lists were examined, and international experts were consulted for relevant published and unpublished work. Papers were subcategorised by anatomical region (trachea, larynx, salivary glands, and tongue or pharynx) and by usage of animal models for in vivo studies. Author, year, country, study type, sample size, mean age, condition, follow-up, intervention type, technique, materials used, main outcome, complication and results were extracted from original human studies.

Primary and secondary outcomes

Primary outcomes from original human studies were: mechan□ical and functional properties of the treated site, integration of grafts, absence of immune response and mortality at the end of follow up. Secondary outcomes were: morbidity, epithelialisa□tion, normal airway function, quality of life, complications, safety and efficiency of preliminary results

Results

Global distribution and categorisation

Of 865 articles found, 149 were excluded because of duplica□tion, replication of a previous study or irrelevance, leaving 716 publications (Figure 2). Institutions in the USA contributed 245 publications; institutions from East Asia and Western Europe also contributed substantial numbers. Figure 3 shows an annotated map that highlights countries that contributed over five publications. Figure 4 subdivides the 716 publications into 158 reviews, 87 conference abstracts and 471 original articles, of which 239 were in vivo, 214 in vitro and 18 were original human studies. Fifteen human studies were interventional, 1 was retrospective and 2 were prospective (Tables 1 and 2). Figures 5 and 6 represent the breakdown of original studies and animal models used for in vivo studies by anatomical region. Figures 7 and 8 represent the techniques and treat□ments used in the human studies included in this review. Within a general year-on-year increase in total publications, 2016 saw the largest number with 72 publications, of which 35 were trachea focused, a similar proportion seen overall (Figure 9). One noticeable dip in 2008 to 2009 coincided with a global economic recession

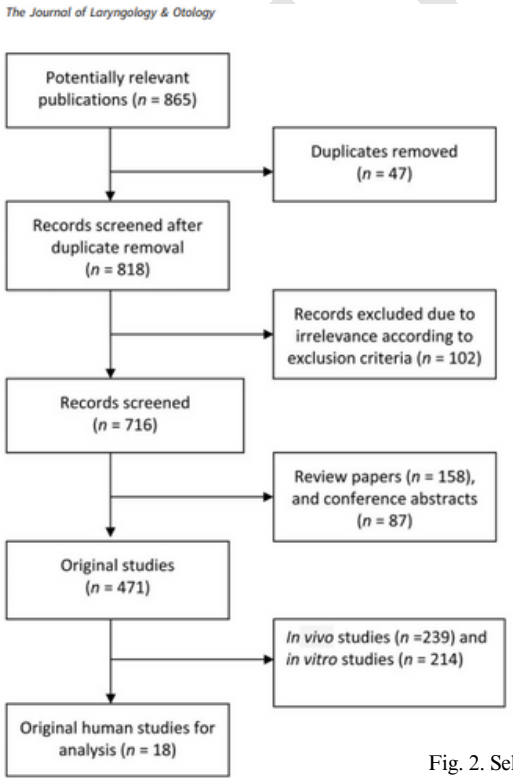

Fig. 2. Selection of articles for further analysis.

Vocal folds

The vocal folds are the functional unit of the larynx. Scar for□mation and fibrosis secondary to abuse, iatrogenic injury, irradiation or trauma may cause vocal loss or change. This review identified two main regenerative strategies to reduce scar□ring: thefirst is inhibition offibrosis and repair of scarring, and the second is regeneration of the extracellular matrix.

Biomaterials for correcting defects require similar viscoelastic mechanics to the natural lamina propria (the vibratory component of vocal folds) and must facilitate tissue regeneration. Through techniques alluded to later, these show potential for addressing vocal fold fibrosis and age-related vocal fold atrophy.

Trachea

Tracheal defects, or congenital or acquired stenosis, may cause variable degrees of airway obstruction. Tracheal resection or aggressive radiotherapy may be necessary for benign or malig nant disease, with resectable lengths under 30 per cent in children or 6 cm in adults. Tissue engineering and stem cell implantation, the most prevalent techniques found, have had mixed success in treating tracheoesophageal fistula, lesions and stenosis

Salivary glands

target of studies attempting to restore salivary Studies have treated irradiated glands with gene editing,34 adipose-derived stem-cells from stromal vascular fraction and have used acellular dermal matrix as an interposition graft post-parotidectomy to target Frey's syndrome

Tongue

Tissue-engineered oral mucosa has been used to repair defects from tongue carcinoma and freeing of the tongue with promising mucosal integration of graft,tongue movability and quality of life indicators

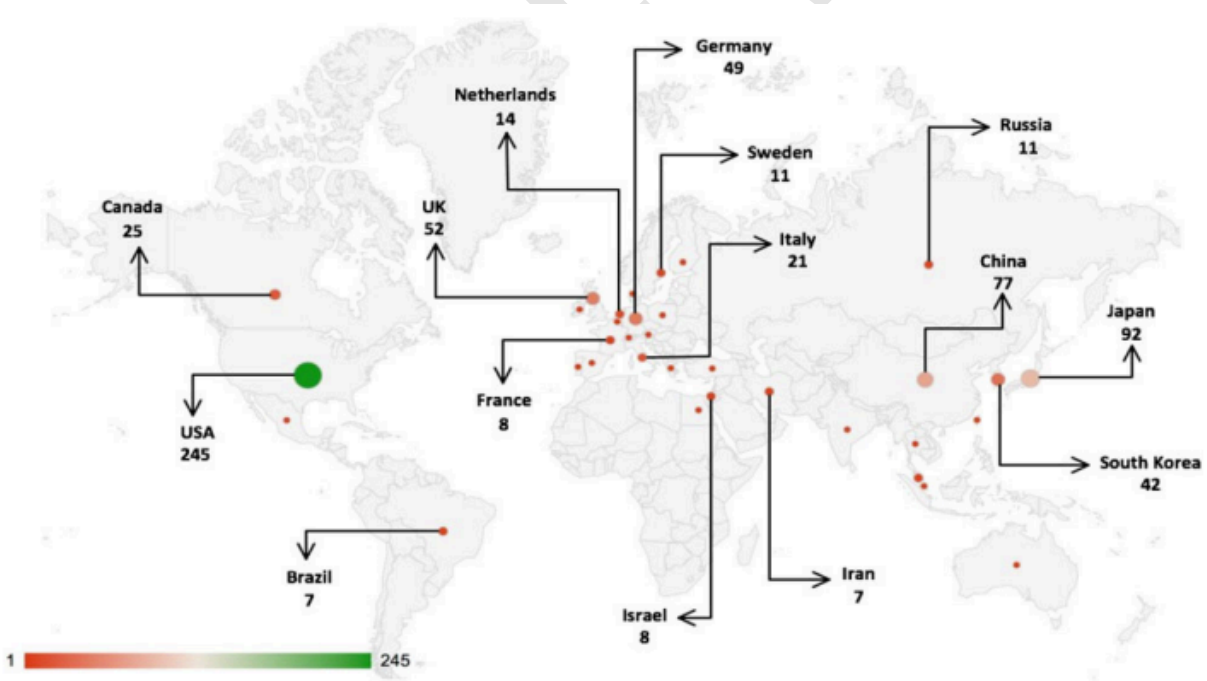

Fig. 3. Annotated map highlighting countries that contributed at least five publications.

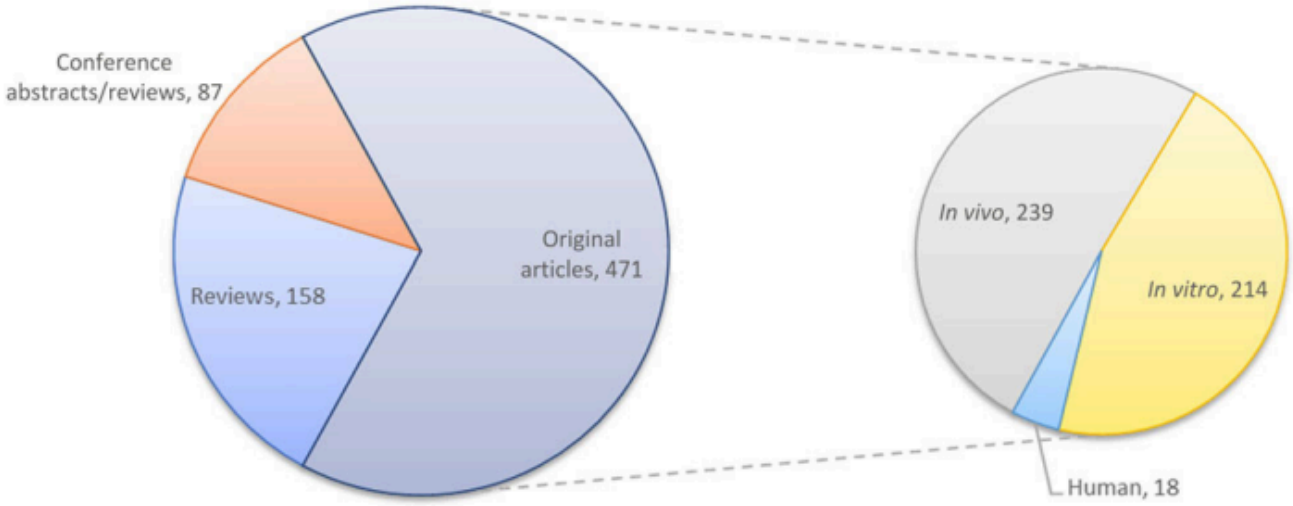

Fig. 4. Categorisation of type of publications reviewed.

Regenerative techniques

The strategies found in human clinical studies over the last two decades are outlined and include: (1) cell transplantation, (2) injection of bioactive factors, (3) scaffolds and (4) bio‐engineering of laryngeal structures.

Cell transplantation

tem cells have been used (Tables 1 and 2). Stem cell based tissue engineering and gene therapy is practised in the optimal cellular microenvironment under the influence of regulatory proteins. Culturing should be strictly monitored to prevent early genetic changes occurring within the stem cells, given that they may undergo carcinogenesis after implantation.

They have been used for autologous tissue generation in grafts and transplantation for vocal fold regeneration, tracheoesophageal fistula, xerostomia, congenital tracheal stenosis and pulmonary sling. For example, in one case report, a bioartificial airway patch (BioVasc, Interfacial Engineering and Biotechnology, Stuttgart, Germany) seeded with autologous microvascular endothelial cells (9 × 106 microvascular endothelial cells) and skeletal muscle cells (6 × 106 skeletal muscle cells) was implanted into a defect extending from the trachea to the left main bronchus following caustic injury. The microvascular endothelial cells were grown under standard conditions (37°C, 5 per cent carbon dioxide) for 4 weeks in endothelial cell growth medium (Promocell, Heidelberg, Germany). The skeletal muscle cells were grown in 4°C Dulbecco's modified Eagle's medium supplemented with 15 per cent foetal calf serum.

Table 1. Basic characteristics of included human studies

Author, year, country	Study type	Sample size (*n*)	Age (mean or range; years)	Condition & treatment	Follow up	Type of treatment	Main outcome measure
Sauerbier *et al.*,[38] 2006, Germany	Intervention	10	53–68	Freeing of the tongue with engineered oral mucosa	6 months	Stem-cell treatment	Histological delineation of the stratum, epithelial crest & a basal membrane
Alvarez *et al.*,[29] 2008, Spain	Intervention	1	67	Tracheoesophageal fistula with adipose-derived stem cells	2 years	Stem-cell treatment	Epithelialisation at base of the fistula, gradual disappearance of adenopathy, deposition of fibrous connective tissue, neoangiogenesis
Hotta *et al.*,[37] 2007, Japan	Intervention	15	66.6	Tongue squamous cell carcinoma (*n* = 2), leukoplakia (*n* = 9), alveolar hypoplasia (*n* = 4), with engineered oral mucosa equivalent	1 year	Stem-cell treatment	Scar formation, laminin & type IV collagen in basement membrane of the implanted sheet; arrangement & positioning of keratinocytes
Macchiarini *et al.*,[28] 2008, Spain	Intervention	1	30	End-stage bronchomalacia with seeded donor trachea	4 months	Stem-cell treatment	Lung function tests, quality of life, mucosalisation & integration/rejection of graft; mechanical properties at 4 months
Omori *et al.*,[33] 2008, Japan	Intervention	4	71.25	Laryngotracheal defects (subglottic stenosis, *n* = 1, & thyroid cancer, *n* = 3) with Marlex mesh scaffold	34 months	Cartilage regeneration techniques	Good epithelialisation on the airway luminal surface & absence of obstruction in post-operative endoscopy at 8–34 months
Ye *et al.*,[36] 2008, China	Intervention	64	42	Parotid tumours: parotidectomy & graft (*n* = 64) *vs* parotidectomy (*n* = 108)	16 months	Acellular interposition graft	Reduction in development of salivary fistula in the treatment group; starch iodine tests performed
Kanemaru *et al.*,[41] 2010, Japan	Intervention	3	51.6	Stenosis of trachea &/or cricoids with Marlex mesh scaffold	2 months & 6 months (staged procedure)	Cartilage regeneration techniques	Sufficient air space in the trachea & cricoid by CT & endoscopy 6 months after the second operation; histological mucosal integration
Chhetri & Berke,[23] 2011, USA	Prospective, pilot	5	55.6	Vocal fold scarring with autologous fibroblasts	12 months	Injection of bioactive factors	Improved mucosal wave grade, voice handicap index & quality of life questionnaire. Safety profile assessed
Elliott *et al.*,[3] 2012, UK	Intervention	1	12	Congenital tracheal stenosis & pulmonary sling with seeded donor trachea	12 months	Stem-cell treatment	Outcomes were survival, morbidity, endoscopic appearance, cytology & proteomics of brushings, & peripheral blood counts
Tamura *et al.*,[24] 2015, Japan	Intervention	6	54.7	Unilateral vocal fold paralysis with b-FGF augmented fat	10 months	Injection of bioactive factors	Volume of injected fat tissue in cases treated using b-FGF compared with conventional fat implantation method, assessed by CT
Steinke *et al.*,[40] 2015, Germany	Intervention	1	26	Tracheoesophageal defect from caustic injury with seeded porcine jejunal scaffold	2.5 years	Cartilage regeneration techniques	Biopsy & bronchoscopic brushings from tracheoesophageal reconstruction with bioartificial airway patch
Alevizos *et al.*,[34] 2017, USA	Intervention	5	57.4	Radiation induced salivary hypofunction with gene transfer	3–4 years	Stem-cell treatments	Relief from 2 key xerostomic symptoms for at least 2 years after treatment, increased parotid flow, immune response, long-term benefit
Ohno *et al.*,[26] 2016, Japan	Prospective intervention	6	68	Age-related vocal fold atrophy with b-FGF injections	6 months	Injection of bioactive factors	Voice handicap index-10, GRBAS scale, phonation time, amplitude & pitch perturbation quotient. Glottic closure & mucosal wave via stroboscopy
Elliott *et al.*,[31] 2017, UK	Intervention	1	15	Critical, refractory tracheal stenosis with cell-seeded tracheal graft	3 weeks	Stem-cell treatments	Early results were encouraging. At 3 weeks, acute intrathoracic bleed caused sudden airway obstruction
Comella & Bell,[35] 2017, South Africa	Intervention	1	54	Radiation-induced xerostomia with stromal vascular fraction/platelet-rich plasma	31 months	Stem-cell treatments & platelet rich plasma	Increase in glandular size at 31 months, increase in saliva production (2–4 mm from baseline)
Kanazawa *et al.*,[25] 2017, Japan	Retrospective	19	60.3	Univocal fold paralysis with b-FGF	6 months	Injection of bioactive factors	Voice handicap index, maximum phonation time, mean airflow rate & pitch range improvement after b-FGF injection
Martinod *et al.*,[32] 2018, France	Intervention	13	54.9	End-stage tracheal & bronchial lesions with aortic allograft transplant of trachea (*n* = 5), of bronchus (*n* = 7), of carina (*n* = 1)	Short: 90 days; long: 3 years 11 months	Stem-cell treatments	Primary outcome: 90-day mortality. Secondary outcome: 90-day morbidity. Regeneration of epithelium & *de novo* generation of cartilage within aortic matrices from recipient cells
Mattei *et al.*,[22] 2018, France	Intervention	1	43	Scarred vocal folds with autologous adipose-derived stromal vascular fraction	1 year	Stem-cell treatments	Stroboscopy, voice handicap index & dysphonia by GRBAS scale

CT = computed tomography; b-FGF = basic fibroblast growth factor; GRBAS = grade, roughness, breathiness, asthenia, strain

Bronchoscopic biopsies confirmed scar-free airway reconstruction, showed seeded cells merged into native airway mucosa and showed no histological evidence of tissue dedifferentiation.

Injection of bioactive factors

Bioactive factors are mainly growth factors that regulate cell proliferation and differentiation. The most studied and clinically successful growth factor is basic fibroblast growth factor (Tables 1 and 2). In one report, 19 subjects were injected with 50 μg basic fibroblast growth factor into the vocal muscle layer. After six months, voice handicap index, maximum phonation time, mean airflow rate and pitch range significantly improved. It is furthermore a relatively routine procedure. In another study for age-related vocal fold atrophy, 10 mcg basic fibroblast growth factor in 0.5 ml of saline was injected trans-orally into the superficial lamina propria on both sides of the vocal folds. Voice handicap index-10 scores, glottal insufficiency and mucosal wave scores all improved.

Scaffolds

Scaffolds involve decellularised organ matrix, biological polymers, synthetic biomimetic hydrogels and synthetic polymers (Tables 1 and 2). Injectable scaffolds have been used for threedimensional vocal fold lamina propria replacement, providing ease of application, requisite biomechanical properties, and delivery of cells and growth factors. They have also modulated the autoinflammatory response and facilitated remodelling of the extracellular matrix elsewhere. Rehydrated and shaped Renovtissue sheets (freeze-dried acellular human dermis; Beijing Qing Yuan Wei Ye Bio-tissue Engineering Company, Beijing, China) successfully reduced salivary fistula rates after superficial or partial parotidectomy (Tables 1 and 2).

Marlex mesh (CR Bard, Billerica, USA) tube scaffolds with collagen sponge have been used for covering tracheal defects secondary to tracheal or subglottic stenosis, airway reconstruction, and thyroid cancer (Tables 1 and 2). Kanemaru et al. used polypropylene Marlex mesh covered with porcine dermal atelocollagen sponge of type I (70 per cent) and type III (30 per cent) collagen, freeze-dried and cross-linked in a vacuum dry oven for several weeks. The scaffold is sutured to the resected or deficient tracheal segment along with venous blood and basic fibroblast growth factor.

Bioengineered laryngeal structures

Decellularised human larynges, infused with growth factors for angiogenesis and neovascularisation, have mechanical properties similar to original tissue. Either cryopreserved allografts or cadaveric tracheal scaffolds have been used (Tables 1 and 2). If refined, these may provide functional partial laryngectomy reconstructive options or scaffold for total laryngeal regeneration.

Discussion

Over 90 per cent of the original studies originated from North America, Western Europe or East Asia, reflecting the global distribution of combined scientific output within the biotechnology sector. Alongside other industries, the centralisation of expertise and resources drives productivity, a positive sign for potential growth in regenerative therapies targeting upper aerodigestive tract disease.

Fifty-one per cent of original studies focused on airway regeneration and replacement. A steeper upward trajectory

Table 2. The impact of interventions on the clinical outcomes in included human studies

Author, year, country	Region of intervention	Technique	Materials used	Cells or factors included	Cell seeding	Complication	Result & conclusions
Sauerbier *et al.*,[38] 2006, Germany	Tongue	Tissue engineering	Oral mucosal biopsy, serum, cell culture medium, trypsin	Blood serum	Keratinocytes transferred to membrane	Minor donor site scarring	Tissue-engineered oral mucosa was applied successfully. Healing time not superior to regular harvested oral mucosa transplants, but a reduction in morbidity
Alvarez *et al.*,[29] 2008, Spain	Trachea	Tissue engineering	Autologous ASC in fibrin glue	Autologous ASC	Direct aspiration & injection to the fistula	No complications reported	ASC can be used safely for the successful treatment of complex tracheomediastinal fistulas. Patient was leading a completely normal life 2 years after stem cell therapy
Hotta *et al.*,[37] 2007, Japan	Oral mucosa	Artificial transplantation	Autologous oral keratinocytes, cultured onto allogenic dermal sheet	Keratinocyte growth supplement-V2*, calcium in culture	Autologous oral keratinocytes seeded as epithelial monolayer	No severe complication	Clinical transplantation of Evpome† sheet resulted in earlier epithelialisation, shorter healing & negligible scar contracture. Keratinocyte-derived cytokines played an important role in the early stages of mucosal wound healing
Macchiarini *et al.*,[28] 2008, Spain	Trachea & bronchus (left)	Tissue engineering	Donor trachea, anti-human monoclonal antibodies	Epithelial cells & mesenchymal stem-cell derived chondrocytes	Bioreactor system	No complications reported	Improved quality of life with normal daily activities; mechanical properties of native airway at 4 months. Full integration of graft with no immunosuppression. Cellular, tissue-engineered airway with mechanical properties that allow normal functioning is possible

Omori *et al.*,[33] 2008, Japan	Larynx & trachea	Tissue engineering	Collagenated Marlex mesh scaffold tube	Plasma	Seeding of autologous venous blood	One case of transient post-operative air leak	In both subglottic stenosis & thyroid cancer, resected region was reconstructed with scaffold successfully. Technique shows great potential for regeneration of airway defects
Ye *et al.*,[36] 2008, China	Parotid glands	Tissue engineering	Renovtissue (freeze-dried acellular human dermis)	Not required	Not required	No complications reported	Reduced salivary fistulas in treatment group (2%) *vs* control group (61%). Acellular dermal matrix as an interposition graft was effective in preventing Frey's syndrome after parotidectomy
Kanemaru *et al.*,[41] 2010, Japan	Trachea	Tissue engineering	Collagenated Marlex mesh scaffold	b-FGF	Not required	No complications reported	Easy breathing & comfortable daily activities. Artificial trachea with b-FGF, a useful biomaterial for tracheal/cricoid stenosis & tracheal defects
Chhetri & Berke,[23] 2011, USA	Vocal folds	Cellular therapies	Punch biopsy, culture media, enzymes	Not required	Three doses of 1–2 × 10^7 cells/ml injected at 4 weekly intervals	Temporary otalgia noted in two subjects	Twelve-month improvement in mucosal wave grade, & VHI questionnaire. Injection of autologous fibroblasts into the scarred vocal fold lamina propria layer is effective & safe
Elliott *et al.*[3] 2012, UK	Trachea	Tissue engineering	Cadaveric tracheal scaffold, autologous bone marrow, cell media	Granulocyte-colony stimulating factor, human recombinant erythropoietin, transforming growth factor β	Total mononuclear cell count: 2.56 × 10^8	Strong local neutrophil response after 8 weeks	Graft revascularised within 1 week. Restoration of epithelium after 1 year. Biomechanical strength developed in 18 months. Normal chest CT & ventilation/perfusion scan at 18 months. Functional airway & normal daily activities at 2 years. Potential for paediatric, stem-cell based, tissue-engineered trachea
Tamura *et al.*[24] 2015, Japan	Vocal folds	Tissue engineering	Autologous fat tissue with b-FGF, gelatine	b-FGF	Not required	No severe complication	Addition of b-FGF in low concentration reduced amount of fat tissue lost to absorption after vocal fold augmentation
Steinke *et al.*,[40] 2015, Germany	Trachea	Tissue engineering	Bioartificial airway scaffold, autologous microvascular endothelial cells & skeletal muscle cells, culture media	Not required	9 × 10^6 autologous microvascular endothelial cells; 6 × 10^6 skeletal muscle cells	No complications reported	At 2.5 year follow up, scar-free reconstruction. Respiratory airway mucosa lining the bronchial reconstruction completely merged into the native airway mucosa. Perfect airway healing, no histological evidence of tissue differentiation
Alevizos *et al.*,[34] 2017, USA	Parotid gland (single)	Cellular therapy	Anti-adenovirus 5 neutralising serum antibodies to block transduction cells by anti-adenovirus 5 vector, adenovirus/cytomegalovirus vector encoding luciferase	Human aquaporin-1 cells	Adenovirus/human aquaporin-1 added to cultures of primary cells from human parotid & minor salivary gland	No severe complication	Marked increase in parotid flow in 3–4 years. Long-term benefits of human aquaporin-1 gene transfer reported. Human aquaporin-1 protein in acinar, but not duct, cell membranes
Ohno *et al.*,[26] 2016, Japan	Vocal folds	Tissue engineering	Human recombinant b-FGF, saline	b-FGF	Not required	No complications reported	VHI-10 score improved, glottic insufficiency & mucosal wave all improved. b-FGF injection is promising for treating age-related vocal fold atrophy
Elliott *et al.*,[31] 2017, UK	Trachea	Tissue engineering	Ricordi chamber & enzymes for decellularisation, Triton X, saline, bone marrow MSCs, culture facility, nasal biopsies, bioreactor	Not required	MSC monolayer: 7.7 × 10^6 cells, epithelial cells: 3500 cells/cm^2	At day 15, narrowing of tracheal graft, acute extrinsic compressive event (probably intrathoracic haemorrhage) caused cerebral hypoxia	Negative outcome of 'compassionate use' case highlights difficulties in clinical translation of preclinical *in vivo* models. Protocols for phase I clinical trials need to be refined. Advised use of stents during the first few months post-transplantation
Comella & Bell,[35] 2017, South Africa	Parotid & submandibular glands	Cellular therapy procedures	Commercial kit for stromal vascular fraction, culture media	Lipoaspirate of fat tissue, plasma	2.5 million adipose-derived stem cells resuspended in 1.5 ml of platelet-rich plasma	No complications reported	Well tolerated procedure. Improved quality of life, willingness to continue treatment, strong safety profile, high efficiency in preliminary results
Kanazawa *et al.*,[25] 2017, Japan	Vocal folds	Tissue engineering	Recombinant b-FGF, saline	b-FGF	Not required	No severe complications	b-FGF significantly improved hoarseness because of unilateral vocal fold palsy. Routine procedure that can be performed in out-patients
Martinod *et al.*,[32] 2018, France	Trachea, bronchi	Tissue engineering	Custom made fully covered conical nitinol stent or silicone stent	Heparin, aerosolised saline solution administered	Not required	One carinal transplant death, 4 major morbidity events unrelated to surgical technique	Carinal transplant death; none for trachea or bronchial (7.7% total mortality). Adverse events (31%) unrelated to surgery. Cryopreserved aortic allografts were viable & could proliferate on thawing. Stented aortic matrices are feasible for complex tracheobronchial reconstruction
Mattei *et al.*,[22] 2018, France	Vocal folds	Cellular therapy	Autologous ADSVF, Ringer's lactate	Good manufacturing practice grade enzymes for digesting ADSVF	Not required	No severe complications	Improvement in parameters of voice assessment (VHI mainly). Therapeutic potential of ADSVF highlighted

*Cascade Biologics, Portland, USA; †AlloDerm, LifeCell, Branchburg, USA. ASC = adipose derived stem cells; b-FGF = basic fibroblast growth factor; VHI = voice handicap index; CT = computed tomography; MSC = mesenchymal stem cell; ADSVF = adipose-derived stromal vascular fraction

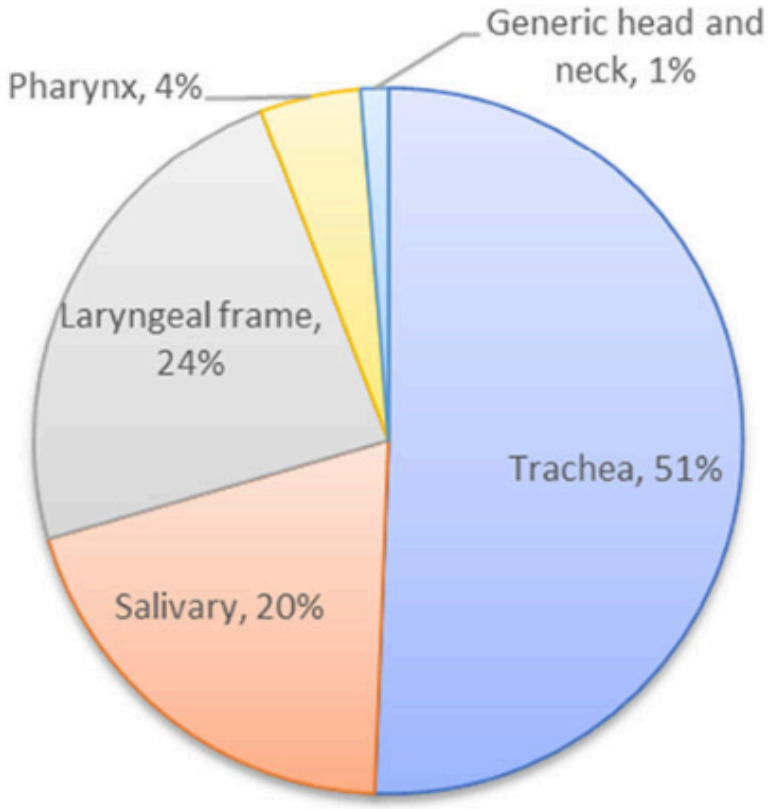

Fig. 5. Distribution of all 471 original studies by anatomical region.

can be observed in airway research compared with other upper aerodigestive tract regions over the past 15 years (Figure 9). Eight of the 18 example human studies reported on airway replacement (trachea or a main bronchus). It is important to separate partial from total airway replacement therapy procedures. In partial tracheal replacement, the native trachea frame is left intact and provides mechanical support for the airway framework. The implant re-vascularises and re-epithelialises from native trachea vessels, countenancing loss of mechanical integrity and delayed epithelialisation, as reported by good functional outcomes and safety profiles. However, total tracheal replacement requires that the replacement construct mechanically withstands the dynamic forces of the entire airway. The lack of distinct tracheal blood supply requires implant pre-vascularisation or a derivation of nutrients from in-growing vasculature from proximal and distal anastomoses.

This review found four human studies reporting total tissue-engineered tracheal or bronchial replacement. Three were single-patient case reports of decellularised tracheal segments repopulated with autologous epithelial cells. One patient died three weeks post-operatively; the other two required long-term stenting and periods of intensive hospital care. The other study used aortic homograft for tracheal replacement. Despite medical complications, no mortality causally related to the surgery was reported, and 80 per cent of recipients were free of airway prostheses after a mean of 18.2 months. Although larger, multicentre trials are required for full effectiveness and safety profiling, the relative success and more mature development stage of aortic homograft as a scaffold for total tracheal or bronchi replacement warrants future targeted research.

Although refractory long-segment tracheal stenosis severely impacts sufferers' life expectancy and quality of life, it is a relatively rare condition. Tracheal stenosis has been treated by tissue-engineered artificial trachea and stem cell based tracheal replacement for congenital tracheal stenosis and pulmonary sling. Artificial tracheal implantation with collagenated Marlex mesh, performed via a staged operation in the presence

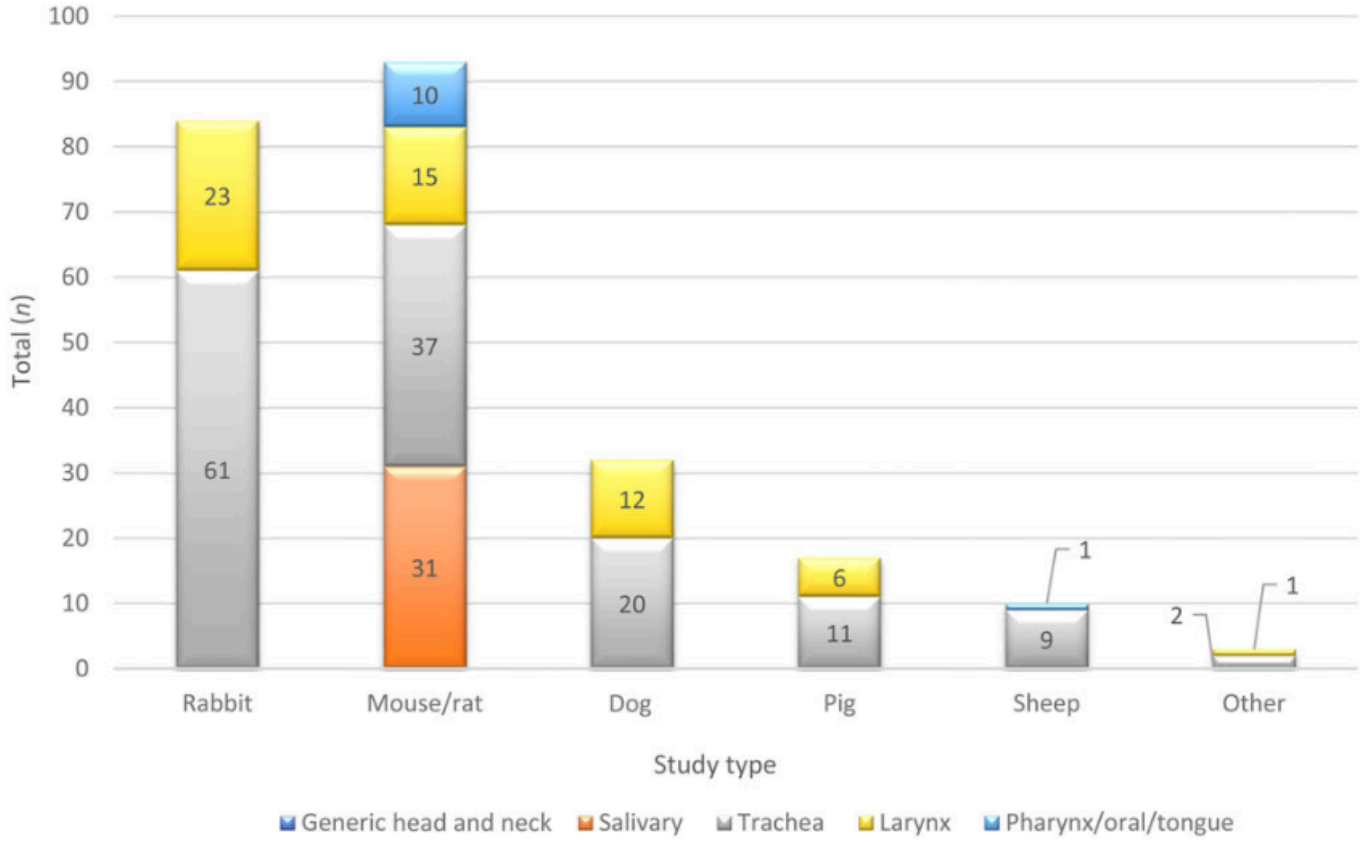

Fig. 6. Breakdown of *in vivo* studies by animal used and anatomical region.

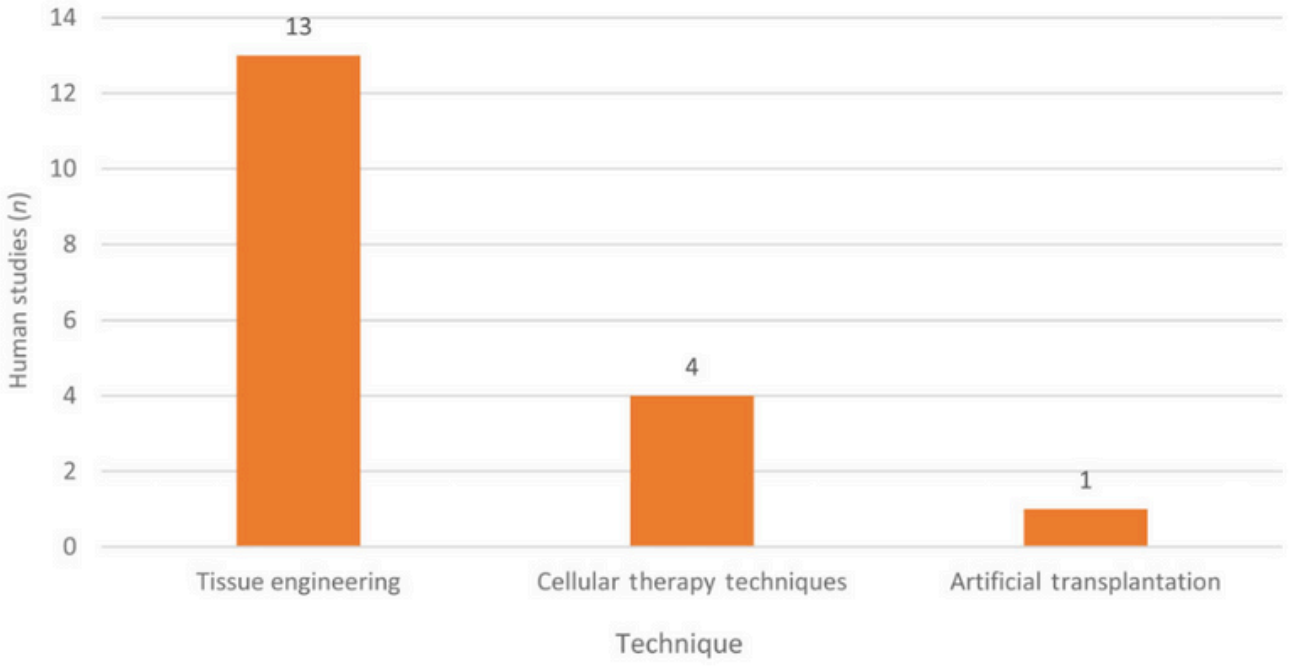

Fig. 7. Techniques used in included human studies.

of basic fibroblast growth factor, has shown promise in treating tracheal stenosis and defects. Patients reported easy breathing and pain-free daily activities.41 In 2012, Elliott et al. reported a decellularised cadaveric tracheal scaffold for congenital tracheal stenosis and pulmonary sling.3 Bone marrow mesenchymal stem cells were seeded (total mononuclear cell counts 2.56×10^8) and grafted with patches of autologous epithelium. Topical human recombinant erythropoietin was applied to encourage angiogenesis and transforming growth factor β for chondrogenesis.

During follow up, the patient developed a strong neutrophil response after eight weeks, generating luminal DNA neutrophil extracellular traps. At two years, the patient had a functional airway and normal daily activities.3 As a paediatric study on a single patient, results are not fully applicable; validation is required with larger samples. In this review of 111 laryngeal framework focused original studies, 80 concerned the vocal folds, and at least 45 primarily concerned the restoration or regeneration of scarred or fibrotic vocal tissue.

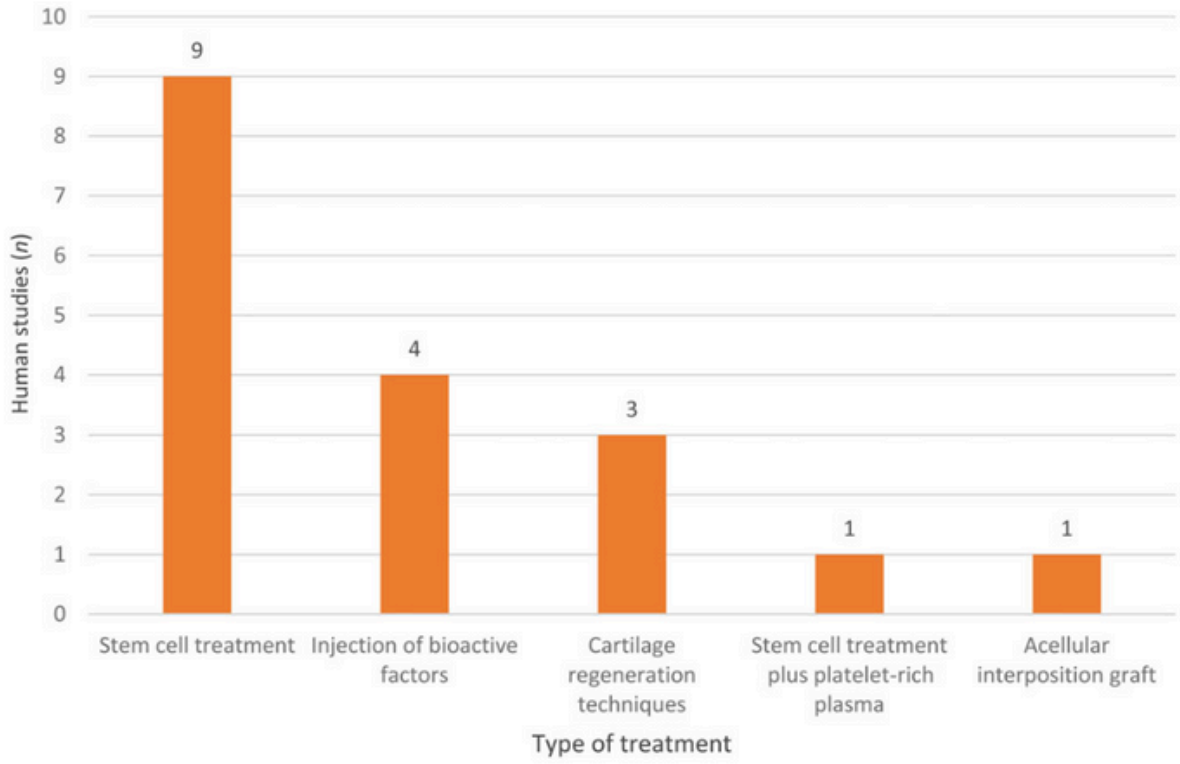

Fig. 8. Types of treatments in included human studies.

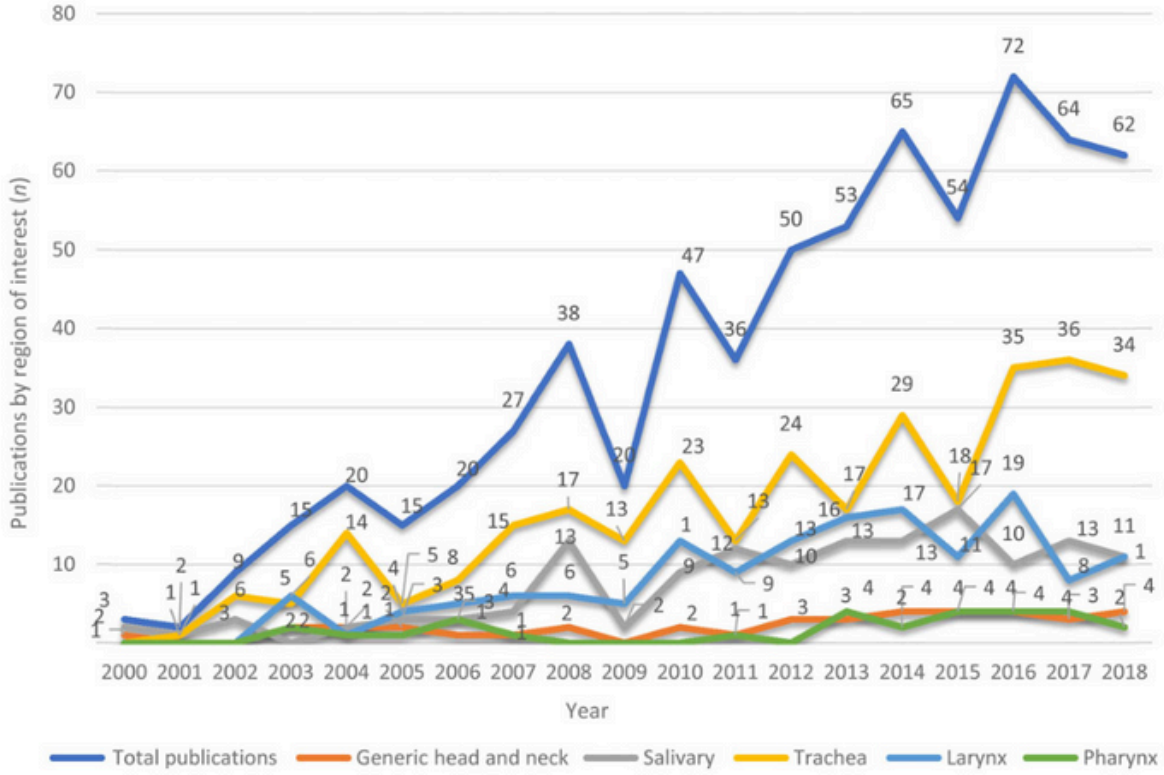

Fig. 9. Timeline of publications.

ligament, preventing effective propagation of the mucosal wave during phonation, inducing chronic hoarseness. This annually affects thousands worldwide and can disastrously impact quality of life. Cell therapies, small molecules and injected biomaterials all saw moderate success in overcoming fibrosis in human studies.

In one case, an autologous-derived stromal vascular fraction was injected locally into scarred vocal fold, resulting in improved voice measurements, an improvement on earlier studies using autologous fat or fascia alone. Another group trialled multiple injections of autologous fibroblasts sourced from buccal mucosa into the lamina propria. Three doses of 1–2 × 107 cells/ml were given at four-weekly intervals. Improvement was seen in voice handicap scores and mucosal wave grade in all five subjects, with minimal complications. Autologous fibroblast injection into scarred lamina propria was well tolerated, although two subjects reported transient otalgia post-treatment. Despite the potential for injectable regenerative therapies to address vocal fold fibrosis, the literature lacks consensus on the optimal cell type, material and delivery method for laryngeal regeneration. Any injectable requires durability and similar viscoelastic properties to the lamina propria. Future studies should target determining the most effective method, which would attract investment around a specific design, streamlining production towards scalable and efficacious novel therapies.

Xerostomia results from hyposalivation and commonly follows radiotherapy for upper aerodigestive tract cancer. Its relatively high incidence and paucity of effective treatments makes it a clear target for regenerative therapy to replace or repair damaged salivary tissue. Twenty per cent of studies in this review focused on the salivary system, although only one human case report and one series of five patients were found that aimed to restore salivary function. Pre-clinical studies broadly divided into studies regenerating salivary tissue in vitro and re-implanting to restore function, and studies that implanted cells, such as adipose-derived stem cells, or used gene editing to stimulate regeneration of existing salivary tissue. The discovery of salivary stem cells that may repair or replace damaged salivary tissue provides another target for regenerative treatments. One example is work by Banh et al. in which activation of aldehyde dehydrogenase 3, a marker of submandibular stem cells, induced an increase in stem cell yield following extraction in a murine model.

Only eight original studies primarily concerned tongue regeneration. This is surprising given fibrosis or loss of tongue volume commonly follows radiotherapy and surgery for upper aerodigestive tract cancer and significantly affects phonation and swallow. Cell therapy procedures and small molecules that can overcome fibrosis, as with the vocal folds, would benefit patients and would be relatively easy to deploy endoscopically as a graft or injectable. Furthermore, the use of biomaterials and tissue engineering to restore muscle bulk would be advantageous in upper aerodigestive tract cancer patients, alongside stroke patients with atrophy following denervation of musculature. Tongue tissue mainly consists of striated muscle that is highly vascular, rendering ex vivo fabrication and implantation more achievable than complex organs with different cellular layers and no distinct blood supply. In 10 cases of freeing of the tongue, oral mucosa was harvested, divided and resuspended in culture media. Cultured keratinocytes were transferred to the membrane (2 × 10 cells/cm2). The procedure was successful; however, healing time and the outcome did not appear to be superior to regular harvested oral mucosa transplants. Regenerative therapies aimed at restoring tongue bulk and overcoming fibrosis are therefore clear additional targets for future research activity.

This review is not without limitations. Firstly, despite many studies pertaining to regenerative medicine or surgery in the upper aerodigestive tract, further analysis was restricted to human studies. Future review should compare the techniques and performance of different animal models for different anatomical sites. Secondly, assessing a risk of bias was not practical in the human studies because of the lack of retrospective designs. Finally, future review should focus on conducting a meta-analysis to observe the impact of techniques on outcome.

Conclusion

This review suggests that a future shift towards developing therapy procedures that address pathologies with a higher disease burden may be warranted, particularly with respect to the upper airways, the oropharyngeal mucosa and tongue. Reconstruction or repair of upper aerodigestive tract defects and restoring function will improve the lives of millions of ailing patients. Regenerative techniques may decrease operating time, eliminate the need of a donor site, improve healing and improve quality of life outcomes. Reconstructing laryngeal structures by tissue engineering allows patients to self-express, breathe unaided and in some instances, withstand the secondary effects of cancer treatment. This field of medicine is highly promising for the upper aerodigestive tract region, and its applications are only limited by our current knowledge. The demand for regenerative medicine will continue as upper aerodigestive tract cancer persists as the sixth most common cancer globally.

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