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REVIEW ARTICLE

Regenerative medicine in otorhinolaryngology

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Abstract

Background: Tissue engineering using biocompatible scaffolds, with or without cells, can permit surgeons to restore structure and function following tissue resection or in cases of congenital abnormality. Tracheal regeneration has emerged as a spearhead application of these technologies, whilst regenerative therapies are now being developed to treat most other diseases within otolaryngology.

Methods and results: A systematic review of the literature was performed using Ovid Medline and Ovid Embase, from database inception to 15 November 2014. A total of 561 papers matched the search criteria, with 76 fulfilling inclusion criteria. Articles were predominantly pre-clinical animal studies, reflecting the current status of research in this field. Several key human research articles were identified and discussed.

Conclusion: The main issues facing research in regenerative surgery are translation of animal model work into human models, increasing stem cell availability so it can be used to further research, and development of better facilities to enable implementation of these advances.

Key words: Regenerative Medicine; Stem Cells; Tissue Engineering; Otorhinolaryngology; Otology; Audiology; Laryngology; Rhinology; Head And Neck Cancer; Reconstructive Surgical Procedures

Introduction

Regenerative medicine and its application to surgical disease have rapidly progressed over the last decade. Regenerative research and subsequent clinical application is now advancing the frontiers of every surgical specialty.[1,2] Advanced tissue engineering using biocompatible scaffolds, with or without cells, permits surgeons to restore or, in the case of congenital abnormalities, establish normal structure and function in: the airways (airway replacement), gastrointestinal tract, hepatobiliary system, myocardium, renal and urinary tract, and integument and musculoskeletal tissues.[3–8] However, important barriers remain prior to full integration into routine healthcare. First-in-human studies need to be extended to larger, more detailed trials; human and physical resources for regenerative medicine need substantial expansion; and social, ethical and legal questions, including those surrounding stem cells, require further exploration.[9]

Regenerative surgery utilises the three-part approach of: cells (stem, progenitor or differentiated), suitable biocompatible matrices (synthetic or biological scaffolds) and appropriate signalling (physical or chemical).[10,11] Tracheal replacement and regeneration has emerged as a spearhead application of these technologies,[10] whilst regenerative therapies are now being developed to treat most common and some rarer diseases within otolaryngology. Here, we summarise the most recent advances for each area.

Materials and methods

We conducted a review of the literature surrounding regenerative medicine and tissue engineering, and their application in ENT surgery. The principle author (JCRW) performed a literature search of Ovid Medline and Ovid Embase databases, using variants of the search terms 'regenerative medicine', 'regenerative surgery', 'stem cells', 'tissue engineering', 'tissue transplantation', 'otology', 'rhinology', 'laryngology', 'head and neck', 'otolaryngology' and 'otorhinolaryngology', from database inception to 15 November 2014. Medical Subject Heading terms were used where available; otherwise, text term searches were incorporated to widen our literature search. We used Boolean operators to refine our search, and included all articles, regardless of date and article type, in the initial search.

Articles were analysed and selected based on relevance to the topic of interest. Articles were excluded if they were not in English language or if material covered was duplicated. Review articles were preferred to original research papers in topics where there was

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more substantial literature, whereas primary research articles were preferred in more innovative areas. The references of relevant articles were inspected for additional material. The authors approached field leaders for further key research developments in the areas of interest. The final list of included articles was decided upon by all authors. Information was included from basic science research, completed early phase clinical trials and ongoing clinical trials (Figure 1).

Results and discussion

Otology and audiological medicine

A prominent aspect of ear disease regenerative medicine is the potential use of stem cells to regenerate irreversible hearing loss in sensorineural disease. Sensorineural hearing loss is a result of inner-ear cochlear dysfunction, and involves the loss of sensory hair cells and/or a secondary degeneration of spiral ganglion neurons. It is the commonest disability in developed countries, with a considerable socioeconomic impact.[11]

Ageing is a major factor in hearing decline,[12] with an estimated 63.1 per cent of individuals aged 70 years and older living with hearing loss in the USA.[13] Hearing loss is associated with a number of health issues, including delayed cognitive skill development, depression,[14] cognitive decline and dementia.[13,15] These issues will become more concerning with population ageing and the increased exposure to loud environments, particularly in the younger demographic.[16]

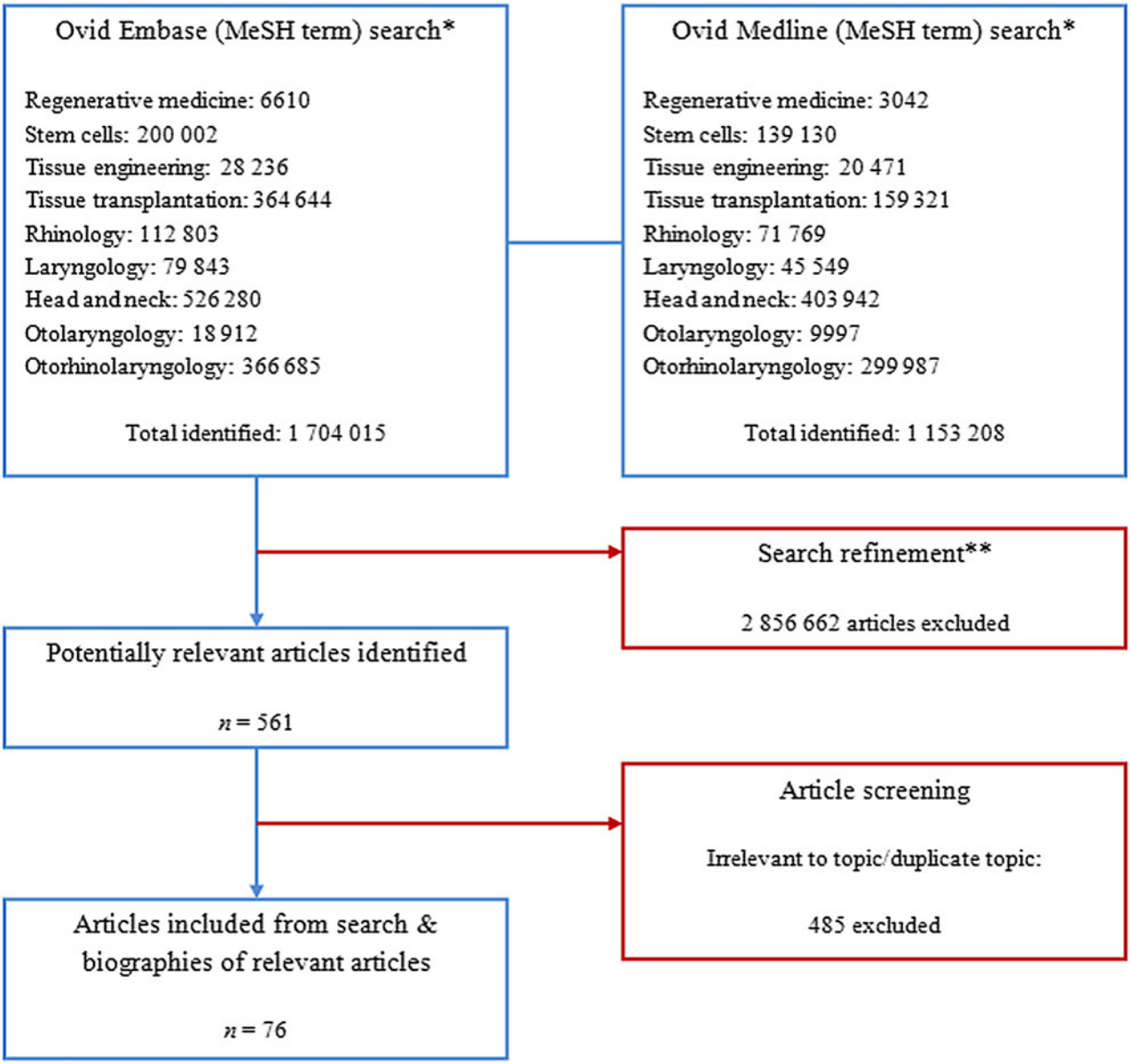

FIG. 1

Article attrition chart. *Medical Subject Heading (MeSH) terms used were: 'otolaryngology', 'nose diseases', 'otorhinolaryngologic diseases', 'larynx', head', 'neck', 'head and neck neoplasms', 'regenerative medicine', 'tissue engineering', 'tissue transplantation' and 'stem cells'. **Search refined with the Boolean operators 'and' and 'or'

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Milestone technological advances, such as cochlear implantation and bone-anchored hearing aids, have radically improved the quality of life for many patients with sensorineural hearing loss. The next step would be the regeneration of neural and cochlear tissue to restore hearing.[17] The two main regenerative strategies currently being researched are: differentiation of endogenous stem cells into new cochlear hair cells, and the introduction of exogenous cells into the inner ear to replace injured hair cells and/or neurons.[11]

The first approach involves inducing resident cochlear cells to proliferate and differentiate into new hair cells and spiral ganglion neurons, to replace lost cells. This would most likely involve targeting stem cells within the inner ear that maintain the ability to differentiate. It is important to note that humans and other mammals do not have the innate ability to regenerate lost sensory cells in the cochlea once development is complete.[18] Therefore, current research efforts have focused on determining the existence of stem cells in the cochlea that could be exploited for endogenous regeneration strategies. Thus far, there has been little evidence that these cells exist in the mature cochlea,[19] although there are some (*in vitro*) data to suggest the presence of a limited number of cells possessing favourable characteristics including limited proliferation capability[20] and expression of adult stem cell markers.[21] Alternatively, there remains the possibility of inducing regeneration by utilising the fully differentiated cells of the inner ear. This could be achieved by: de-differentiating the cells into a more naive state normally only found during development, in order to regenerate sensory cells; direct transdifferentiation of the cells to the target cell type of interest; or using reprogramming strategies.[22]

The second approach involves the transplantation of exogenous cells, and can take advantage of an array of stem cell sources, such as embryonic stem cells, induced pluripotent stem cells, mesenchymal stem cells from the bone marrow, or adipose tissue and amniotic fluid-derived stem cells. There are a number of challenges to this approach. First, there is the potential tumourigenic risk that is intrinsic to all cell-based therapies. Second, there is the issue of controlling differentiation of these cells to ensure that the sensory cells are produced without contamination from other cell types that could interfere with the transplantation of the sensory cells or the function of the organ. Finally, there is the issue of ensuring the survival and integration of exogenous cells into the cochlear sensory epithelium, given the relatively hostile biochemical and immunological environment, the complex architecture of the organ of Corti, and the repair mechanisms of the cochlea, which result in closure of any lesions in the sensory epithelium that arise from hair cell loss. There is also the added technical challenge of delivering the cells to the tissue, although a surgical approach could be used to expose the cochlea in order to implant or inject therapeutic cells into the perilymph (via the scala vestibuli and scala tympani), the endolymph (in the scala media) and the modiolus.[23] A number of promising studies have investigated how various stem cells could be differentiated into sensorineural cells.[24–26] However, the research is still in its early stages and translation to the clinical setting is distant whilst the above issues are still being addressed.

The generation of new hair cells and auditory neurons is particularly challenging because of the importance of hair cell alignment and tonotopic organisation. Regenerated hair cells must be correctly orientated within the sensory epithelium to restore functionality, and must be orientated in the same polarity as native hair cells to transduce relevant stimuli. Auditory hair cells vary in terms of biophysiology depending on their position along the tonotopic axis of the cochlea, and thus newly generated cells must reflect the location-dependent properties of native cells.[27]

The *in vitro* generation of hair-cell-like cells and auditory neurons from embryonic stem cells, induced pluripotent stem cells and fetal auditory stem cells has been a promising area of research.[28] Most recently, Koehler *et al*. created a stepwise protocol differentiating inner-ear sensory epithelia from mouse embryonic stem cells in three-dimensional (3D) culture.[25] Using an *in vivo* 3D culture in combination with bone morphogenetic protein activation and transforming growth factor β inhibition, they stimulated sequential formation of sensory epithelia (non-neural, pre-placodal then otic placode-like epithelia). The sensory epithelia with hair cells possessed functional and structural properties of native hair cells. Koehler's paper highlights the most recent advance in stem cell therapy to treat sensorineural deafness. However, there is a considerable amount of research into the use of genetic and molecular therapies for this condition.[27]

Surgical intervention to treat middle-ear diseases can disrupt the middle-ear mucosa (an epithelium that serves to maintain a functional middle-ear environment), which can lead to retraction of the tympanic membrane causing further disease or even hearing loss. Little research has been carried out to address this issue. The most promising potential intervention appears to be the use of transplanted cells to replace the lost mucosal epithelium.[29,30] For ossicle reconstruction, prosthetics are typically used, but there has been some work looking at using tissue engineering for this purpose, which involves culturing mesenchymal stem cells under osteogenic conditions on bioresorbable 3D scaffolds.[31,32]

Repair of tympanic membrane perforation is an important target for the use of regenerative techniques, especially in chronic perforation. Whilst the majority of the research is based on animal models of acute tympanic membrane perforation, regenerative therapy would probably be targeted at patients with chronic perforation, where currently available interventions have already failed.[33] Of note, Kanemaru *et al*. randomised 63 patients with chronic tympanic membrane

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perforations to receive basic fibroblast growth factor on a gelatine sponge with fibrin glue, or gelatine sponge plus fibrin glue alone.[34] The biomolecular intervention significantly improved healing rates and mean hearing level compared to controls. Other biomolecules under current investigation include hyaluronic acid, epidermal growth factor and platelet-derived growth factor.[33]

Bioscaffolds, such as acellular dermis, have been used to repair the tympanic membrane. A randomised, unblinded trial of acellular dermis versus temporalis fascia for tympanoplasty found that acellular dermis was associated with shorter operative time and less post-operative pain.[35] This trial was limited by methodology and sample size ($n = 42$), but is one of few human trials using this technique at the time of writing.

There is a relative paucity of evidence for the use of stem cells in tympanic membrane perforation. There are a number of key studies in animal models that have shown promise. Rahman and colleagues applied mesenchymal stem cells to rats with chronic tympanic membrane perforations and found that the stem cells enhanced healing and reduced the stiffness of the healed tympanic membrane, leading to enhanced restoration.[36]

Outer-ear reconstruction typically uses autologous costal cartilage grafts from the patient. This method involves multistage surgery and permanent loss of cartilage from the donor site. A good alternative would be a stem cell source combined with a compatible scaffold to mimic cartilage.[37] Recent work using adipose-derived stem cells taken from abdominal tissue, grown on a polyhedral oligomeric silsesquioxane modified polycarbonate urea-urethane ('POSS-PCU') bioscaffold, shows great promise for clinical use.[38] If cost-effectiveness, therapeutic equivalence and safety can be demonstrated, tissue engineering approaches may provide longer-lasting, more effective repairs, without the need to create a donor site. However, although the results of many studies are encouraging, more human clinical trials are required that directly compare tissue engineering with conventional (autologous grafting) techniques. Such techniques may be especially useful in revision cases where autologous graft material may be lacking.

Rhinology

Unlike in otology and laryngology, rhinology plays a greater role in the provision of stem cells rather than in the receipt. The nose contains several populations of relatively easy to access autogenous stem cells that have great potential for use in the regeneration of nerve and cartilage elsewhere. In the olfactory epithelium, olfactory receptor neurons are supported by olfactory ensheathing cells, a form of glial cell with the potential to support neural regeneration within the olfactory epithelium[39] and when transplanted into other tissues such as the brain and spinal cord.[40–42] De Corgnol and colleagues investigated the *in vivo* use of olfactory ensheathing cells in rats to assist surgical reinnervation of the vocal folds following vagus nerve section.[43] Rats with the olfactory ensheathing cell intervention had improved reinnervation results compared to controls in terms of post-operative vocal fold function.

Choi *et al*. prospectively analysed nasal biopsies taken during transsphenoidal surgery.[44] This revealed that the nasal septal mucosa, particularly the superior-posterior area, was a rich source of olfactory ensheathing cells. There has also been some suggestion of the existence of a mesenchymal stem cell population in the olfactory epithelium[45,46] and respiratory epithelium.[47] The nasal septum is also rich in chondrogenic cells. Cartilage is a much sought after tissue in the wider regenerative surgery field, with particular prominence in regenerative orthopaedics procedures, and especially in reconstruction of the airways.[10,48,49] In addition, chondrogenic cells harvested from the cranium and airways are capable of healing, unlike adult musculoskeletal cartilage.

In a recent observational first-in-human clinical trial, five patients received tissue-engineered cartilage implants for nasal reconstructive surgery.[50,51] The investigators reported restoration of contour and nasal airflow in all five patients studied. In addition, at 12 months, no adverse events were recorded, and patients were satisfied with the aesthetic and functional outcomes. Similar composite tissue-engineered tissues are likely to be useful in situations where other options have been exhausted and where tissue for reconstruction is lacking.

Laryngology

Regenerative medicine for laryngology, to replace parts or the whole of the airway, oesophagus and larynx, requires a much larger scale that is best suited to a tissue engineering approach. A combination of a scaffold with the right physical and functional properties, seeded with cells that promote regeneration and allow function, that can be used for transplanting or grafting, has been the primary aim.

Airway reconstruction is indicated in patients with extensive tracheal stenosis following malignancy, trauma or in cases of congenital malformations.[4] In cases where tracheal resection and primary anastomosis is not possible, such as after extensive burns, trauma or tumour resection, tracheal replacement may be indicated.[52] Both cadaveric and synthetic tracheal transplants have been used, but both convey considerable morbidity, including foreign body reactions and fibrotic stenosis. Fresh, viable tracheal allografts usually require considerable immunosuppressive therapy; they are rapidly rejected by the host without such therapy, leading to necrosis and fibrotic stenosis.[53]

In 2008, the first stem cell based tissue-engineered neo-trachea was successfully utilised in a patient.[54] The trachea was generated by an *in vitro* tissue engineering process using a donor trachea, which was decellularised, then readily colonised by the recipient's epithelial cells and mesenchymal stem cell derived chondrocytes. Importantly, the nature of the patient's disease in this case made *in vitro* (thus delayed) preparation of the tissue-engineered organ possible with subsequent implantation. Five-year follow-up data have recently

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been reported, indicating positive long-term outcomes.[55] Following this success, Elliot and colleagues utilised an *in vivo* approach to develop a tissue-engineered neo-trachea for a child requiring emergent airway reconstruction.[10] By using a decellularised cadaveric trachea seeded with autologous mesenchymal stem cells and exposed to topical biochemical inducers of differentiation, the airway was successfully reconstructed with total functionality at two years post-operation.

These significant advances in the generation of stem cell derived airway grafts and the development of advanced bioreactors has led to frontier work on the construction of a tissue-engineered larynx.[56] The larynx, unlike the trachea, is a dynamic organ. This adds considerable complexity to the generation of laryngeal reconstructions, something that is also observed in the reconstruction of other dynamic organs, such as the oesophagus. A neo-larynx therefore requires functional muscle tissue with appropriate reinnervation.

Decellularised skeletal muscle matrices have been described as a potential scaffold for the generation of the muscular activity required for an engineered larynx.[57,58] Fishman *et al.* implanted decellularised laryngeal muscle scaffolds into a rabbit model to determine the *in vivo* effects on scaffold biodegradation time and immunogenicity following implantation.[57,58] They found that their decellularisation process resulted in total DNA clearance and down-regulation of major histocompatibility complex expression, whilst maintaining the scaffold's structural integrity. This improved the longevity of the xenogeneic bioscaffold by reducing the cell-mediated immune response, which also increased its neo-angiogenic potential. The in situ implantation of such a scaffold in combination with stem cells and growth factors has been described in the reconstruction of vocal folds in an animal model.[59] This has yielded promising results in terms of graft survival, functionality and safety, but further research in humans is warranted.

The use of human stem cells as a direct therapy to the larynx, with a view to enhanced healing, is another promising area of research. A small animal study ($n = 12$) that used injectable mesenchymal stem cells which improved vocal fold healing in rabbits found better viscoelasticity of the folds and enhanced healing following injury.[60]

Ultimately, our understanding of stem cell biology, biomaterials and transplantation immunobiology could lead to airway transplantation without systemic immunosuppression. This, along with new technologies such as 3D printing, a novel technique with the potential to render microscopic control over how cells are incorporated and grown onto the tissue-engineered airway, will have a significant impact on patients with airway disease in the future.[56,61]

Head and neck

The loss of salivary gland function can result from pharmacological intervention, surgical resection, radiotherapy and autoimmune diseases. It leads to xerostomia, which in turn may predispose an individual to infections, dysphagia and oral mucosal infections. Xerostomia is usually managed with synthetic saliva supplementation.

There has been considerable research into the development of salivary gland regeneration, with an emphasis on using direct stem cell therapy to heal and regenerate the glands following radiotherapy for head and neck cancer, which is a common cause of xerostomia in the developed world.[62,63] There is comparatively less research to date on the use of tissue engineering techniques used to develop neo-organs to solve this clinical conundrum.

In 2007, Joraku and colleagues successfully regenerated fully functional salivary glands from a single human salivary gland cell using 3D tissue engineering methods *in vitro*.[64] However, at the time of writing, this has not been translated into clinical use. In contrast, the use of direct cellular therapy appears to have had more promising results. Xiong and colleagues exposed rats to radiotherapy to replicate this pathology, and then injected human adipose-derived stem cells into their submandibular salivary glands.[65] The intervention improved salivary gland flow rate and post-radiotherapy healing compared to controls. These results have been replicated in other studies, but advances in humans remain speculative.[66,67]

Tissue engineering for the regeneration of neuronal tissue has been applied to several conditions affecting the head and neck, most notably in the regeneration of the facial and recurrent laryngeal nerves. Disease of the facial nerve is usually treated operatively by autologous nerve grafting. However, tissue engineering approaches are currently under investigation, including the use of induced pluripotent stem cells, mesenchymal stem cells and Schwann cells for regeneration of the facial nerve. Research in this field is currently limited to animal models.

Watanbe *et al.* utilised adipose-derived stem cells, retrieved during liposuction, to regenerate a 7 mm gap in the rat facial nerve, and compared this technique directly with autologous grafting.[68] Some of the adipose-derived stem cells were differentiated into Schwann-like cells and some remained undifferentiated. The adipose-derived stem cells were then embedded onto a collagen scaffold and implanted into the facial nerve defect. Functional recovery was similar for both groups, and both differentiated and undifferentiated adipose-derived stem cells were capable of neuroregeneration.[68]

Basic fibroblast growth factor promotes the regeneration of peripheral nerve defects by affecting nerve cells, Schwann cells and fibroblasts, and increasing the rate of nerve regeneration, the number of regenerated nerves and the degree of regenerated nerve maturation.[69] Cui *et al.* utilised a functional nerve conduit, consisting of a collagen scaffold plus the neurocytokines ciliary-derived neurotropic factor and basic fibroblast growth factor, to repair a considerably larger facial nerve gap

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of 35 mm in a mini-pig model.[70] Their rationale for this model was that nerve injuries in humans are often more substantial than what can be replicated in the rat model. At six months post-implantation, they found that the combination of ciliary-derived neurotropic factor and basic fibroblast growth factor promoted facial nerve regeneration (rather than single neurocytokines) in terms of the number of neurons, myelination and functionality.[70]

Treatment of recurrent laryngeal nerve (RLN) palsy is also under scrutiny from a regenerative medicine point of view. There is evidence that the RLN can spontaneously regenerate following trauma, although the exact process by which this occurs is not fully understood.[71] Halum *et al*. conducted a trial of ciliary-derived neurotropic factor secreting mesenchymal stem cells to attempt to promote this phenomenon.[72] Immunohistochemical analysis identified that the mesenchymal stem cells in combination with neurotropic factor selectively enhanced reinnervation and motor neuron regeneration, although the functional outcome on laryngeal muscle was unclear from that study.[72]

The use of intravenous stem cell therapy, usually mesenchymal stem cells, has shown promise for the regeneration of neuronal tissue following stroke and spinal cord injury. Lerner *et al*. performed a pilot randomised trial of intravenous mesenchymal stem cell use to assess RLN recovery following injury in 12 rats.[73] They found no significant difference between the mesenchymal stem cell group and the control group, although the data were promising for enhanced functional recovery in the experimental arm.

The reconstruction of mandibular defects presents a challenging problem for head and neck surgeons, with current treatment strategies focusing on microvascular free flaps, usually based on the fibula or radius and their respective vascular pedicles. The nature of mandibular defects, usually a result of malignancy, osteonecrosis or trauma, means that the vascular supply of the surrounding tissue is often compromised.[62] Regenerative techniques, such as the *in vitro* culture of tissue-engineered mandibular grafts and the *in vivo* use of cell-signalling factors to stimulate new bone growth *de novo*, are currently under investigation.

A small case series of patients ($n = 14$) underwent mandibular reconstruction with bone morphogenetic protein-2 (a bone-promoting cytokine) in a collagen matrix carrier as an alternative to autologous bone graft.[74] All 14 patients developed new bone tissue at the graft site, which was palpable by 3 to 4 months and radiographically evident at 6 months. That study supports the use of regenerative techniques for mandibular reconstruction, although this technique is only suitable for large mandibular defects.

Lee and colleagues developed bespoke synthetic mandibular bioscaffold grafts using 3D printing techniques, which were then infiltrated with rat adipose-derived stem cells and/or bone morphogenetic protein-2.[75] These grafts were subsequently implanted into 28 rats with large mandibular defects. The authors found that the bioscaffolds with adipose-derived stem cells, bone morphogenetic protein-2 infiltration and both in combination were capable of producing bone regeneration. There are concerns regarding the risk of malignancy following the use of bone morphogenetic protein, as with many cellular therapies. However, interestingly, a large retrospective series of spinal surgery patients in the USA found no increased risk of cancer following bone morphogenetic protein use, contradicting previous findings.[76]

Conclusion

Regenerative approaches can be readily applied to ENT diseases, especially where conventional interventions are limited. Each subspecialty of ENT is developing novel regenerative interventions based on the principles of tissue engineering and cellular therapy. Apart from the main areas of research discussed in this review, there is ongoing work on the use of stem cells in head and neck reconstruction, and in a variety of related surgical specialties. However, despite the advancements in the use of stem cell therapy to restore hearing loss secondary to inner-ear disease, currently little is known about the potential for stem cell therapy in vestibular system diseases, such as Ménière's disease and vestibular neuritis.

The main issues currently facing research in regenerative surgery are translation of animal model work into human models, increasing the availability of stem cells that can be used to further research, and development of better facilities to improve implementation of these advances. Otorhinolaryngology provides some of the most complex challenges for regenerative medicine; these include attaining stem cells from the inner ear for further research and reconstructing the complex anatomy of the larynx using vocal fold scaffolds. This makes regenerative medicine a particularly progressive and dynamic area within ENT surgery.

References

1 Orlando G, Baptista P, Birchall M, De Coppi P, Farney A, Guimaraes-Souza NK *et al*. Regenerative medicine as applied to solid organ transplantation: current status and future challenges. *Transpl Int* 2011;**24**:223–32
2 Orlando G, Wood KJ, De Coppi P, Baptista PM, Binder KW, Bitar KN *et al*. Regenerative medicine as applied to general surgery. *Ann Surg* 2012;**255**:867–80
3 Grikscheit TC, Siddique A, Ochoa ER, Srinivasan A, Alsberg E, Hodin RA *et al*. Tissue-engineered small intestine improves recovery after massive small bowel resection. *Ann Surg* 2004;**240**:748–54
4 Lange P, Fishman JM, Elliott MJ, De Coppi P, Birchall MA. What can regenerative medicine offer for infants with laryngotracheal agenesis? *Otolaryngol Head Neck Surg* 2011;**145**:544–50
5 Maher B. Tissue engineering: how to build a heart. *Nature* 2013;**499**:20–2
6 Shaker A, Rubin DC. Stem cells: one step closer to gut repair. *Nature* 2012;**485**:181–2
7 Totonelli G. Esophageal tissue engineering: a new approach for esophageal replacement. *World J Gastroenterol* 2012;**18**:6900–7
8 Totonelli G, Maghsoudlou P, Garriboli M, Riegler J, Orlando G, Burns AJ *et al*. A rat decellularized small bowel scaffold that

https://doi.org/10.1017/S0022215115001577 Published online by Cambridge University Press

preserves villus-crypt architecture for intestinal regeneration. *Biomaterials* 2012;**33**:3401–10
9 Vandewoude S, Rollin BE. Practical considerations in regenerative medicine research: IACUCs, ethics, and the use of animals in stem cell studies. *ILAR J* 2010;**51**:82–4
10 Elliott MJ, De Coppi P, Speggiorin S, Roebuck D, Butler CR, Samuel E *et al*. Stem-cell-based, tissue engineered tracheal replacement in a child: a 2-year follow-up study. *Lancet* 2012; **380**:994–1000
11 Hu Z, Ulfendahl M. The potential of stem cells for the restoration of auditory function in humans. *Regen Med* 2013;**8**:309–18
12 Roth TN, Hanebuth D, Probst R. Prevalence of age-related hearing loss in Europe: a review. *Eur Arch Otorhinolaryngol* 2011;**268**:1101–7
13 Lin FR. Hearing loss and cognition among older adults in the United States. *J Gerontol A Biol Sci Med Sci* 2011;**66**:1131–6
14 Monzani D, Galeazzi GM, Genovese E, Marrara A, Martini A. Psychological profile and social behaviour of working adults with mild or moderate hearing loss. *Acta Otorhinolaryngol Ital* 2008;**28**:61–6
15 Lin FR, Metter EJ, O'Brien RJ, Resnick SM, Zonderman AB, Ferrucci L. Hearing loss and incident dementia. *Arch Neurol* 2011;**68**:214–20
16 Daniel E. Noise and hearing loss: a review. *J Sch Health* 2007; **77**:225–31
17 Ibekwe TS, Ramma L, Chindo BA. Potential roles of stem cells in the management of sensorineural hearing loss. *J Laryngol Otol* 2012;**126**:653–7
18 Rubel EW, Furrer SA, Stone JS. A brief history of hair cell regeneration research and speculations on the future. *Hear Res* 2013;**297**:42–51
19 Okano T, Kelley MW. Stem cell therapy for the inner ear: recent advances and future directions. *Trends Amplif* 2012;**16**:4–18
20 Rask-Andersen H, Boström M, Gerdin B, Kinnefors A, Nyberg G, Engstrand T *et al*. Regeneration of human auditory nerve. In vitro/in video demonstration of neural progenitor cells in adult human and guinea pig spiral ganglion. *Hear Res* 2005;**203**:180–91
21 Chai R, Xia A, Wang T, Jan TA, Hayashi T, Bermingham-McDonogh O *et al*. Dynamic expression of Lgr5, a Wnt target gene, in the developing and mature mouse cochlea. *J Assoc Res Otolaryngol* 2011;**12**:455–69
22 Jopling C, Boue S, Izpisua Belmonte JC. Dedifferentiation, transdifferentiation and reprogramming: three routes to regeneration. *Nat Rev Mol Cell Biol* 2011;**12**:79–89
23 Pau H, Fagan P, Oleskevich S. Locating the scala media in the fixed human temporal bone for therapeutic access: a preliminary study. *J Laryngol Otol* 2006;**120**:914–15
24 Chen W, Jongkamonwiwat N, Abbas L, Eshtan SJ, Johnson SL, Kuhn S *et al*. Restoration of auditory evoked responses by human ES-cell-derived otic progenitors. *Nature* 2012;**490**: 278–82
25 Koehler KR, Mikosz AM, Molosh AI, Patel D, Hashino E. Generation of inner ear sensory epithelia from pluripotent stem cells in 3D culture. *Nature* 2013;**500**:217–21
26 Oshima K, Shin K, Diensthuber M, Peng AW, Ricci AJ, Heller S. Mechanosensitive hair cell-like cells from embryonic and induced pluripotent stem cells. *Cell* 2010;**141**:704–16
27 Geleoc GS, Holt JR. Sound strategies for hearing restoration. *Science* 2014;**344**:1241062
28 Chen W, Johnson SL, Marcotti W, Andrews PW, Moore HD, Rivolta MN. Human fetal auditory stem cells can be expanded in vitro and differentiate into functional auditory neurons and hair cell-like cells. *Stem Cells* 2009;**27**:1196–204
29 Yaguchi Y, Wada K, Uchimizu H, Tanaka Y, Kojima H, Moriyama H. Middle ear mucosa regeneration by grafting of artificial mucosa. *Acta Otolaryngol* 2007;**127**:1038–44
30 Wada K, Tanaka Y, Kojima H, Inamatsu M, Yoshizato K, Moriyama H. In vitro reconstruction of a three-dimensional middle ear mucosal organ and its in vivo transplantation. *Acta Otolaryngol* 2006;**126**:801–10
31 Danti S, D'Alessandro D, Pietrabissa A, Petrini M, Berrettini S. Development of tissue-engineered substitutes of the ear ossicles: PORP-shaped poly(propylene fumarate)-based scaffolds cultured with human mesenchymal stromal cells. *J Biomed Mater Res A* 2010;**92**:1343–56
32 Luers JC, Beutner D, Huttenbrink KB. Reconstruction of the ossicular chain – current strategies [in German]. *Laryngorhinootologie* 2010;**89**:172–8
33 Hong P, Bance M, Gratzer PF. Repair of tympanic membrane perforation using novel adjuvant therapies: a contemporary review of experimental and tissue engineering studies. *Int J Pediatr Otorhinolaryngol* 2013;**77**:3–12
34 Kanemaru S, Umeda H, Kitani Y, Nakamura T, Hirano S, Ito J. Regenerative treatment for tympanic membrane perforation. *Otol Neurotol* 2011;**32**:1218–23
35 Raj A, Sayal A, Rathore PK, Meher R. Sutureless tympanoplasty using acellular dermis. *Am J Otolaryngol* 2011;**32**:96–9
36 Rahman A, Olivius P, Dirckx J, Von Unge M, Hultcrantz M. Stem cells and enhanced healing of chronic tympanic membrane perforation. *Acta Otolaryngol* 2008;**128**:352–9
37 Nayyer L, Patel KH, Esmaeili A, Rippel RA, Birchall M, O'Toole G *et al*. Tissue engineering: revolution and challenge in auricular cartilage reconstruction. *Plast Reconstr Surg* 2012; **129**:1123–37
38 Guasti L, Vagaska B, Bulstrode NW, Seifalian AM, Ferretti P. Chondrogenic differentiation of adipose tissue-derived stem cells within nanocaged POSS-PCU scaffolds: a new tool for nanomedicine. *Nanomedicine* 2014;**10**:279–89
39 Au E, Roskams AJ. Olfactory ensheathing cells of the lamina propria in vivo and in vitro. *Glia* 2003;**41**:224–36
40 Lindsay SL, Riddell JS, Barnett SC. Olfactory mucosa for transplant-mediated repair: a complex tissue for a complex injury? *Glia* 2010;**58**:125–34
41 Lipson AC, Widenfalk J, Lindqvist E, Ebendal T, Olson L. Neurotrophic properties of olfactory ensheathing glia. *Exp Neurol* 2003;**180**:167–71
42 Vats A, Birchall M. Stem cells and regenerative medicine: potentials and realities for rhinology. *Rhinology* 2010;**48**: 259–64
43 de Corgnol AC, Guerout N, Duclos C, Verin E, Marie JP. Olfactory ensheathing cells in a rat model of laryngeal reinnervation. *Ann Otol Rhinol Laryngol* 2011;**120**:273–80
44 Choi D, Li D, Law S, Powell M, Raisman G. A prospective observational study of the yield of olfactory ensheathing cells cultured from biopsies of septal nasal mucosa. *Neurosurgery* 2008;**62**:1140–4; discussion 1144–5
45 Murrell W, Féron F, Wetzig A, Cameron N, Splatt K, Bellette B *et al*. Multipotent stem cells from adult olfactory mucosa. *Dev Dyn* 2005;**233**:496–515
46 Nivet E, Vignes M, Girard SD, Pierrisnard C, Baril N, Devèze A *et al*. Engraftment of human nasal olfactory stem cells restores neuroplasticity in mice with hippocampal lesions. *J Clin Invest* 2011;**121**:2808–20
47 Jakob M, Hemeda H, Janeschik S, Bootz F, Rotter N, Lang S *et al*. Human nasal mucosa contains tissue-resident immunologically responsive mesenchymal stromal cells. *Stem Cells Dev* 2010;**19**:635–44
48 Gómez-Barrena E, Rosset P, Müller I, Giordano R, Bunu C, Layrolle P *et al*. Bone regeneration: stem cell therapies and clinical studies in orthopaedics and traumatology. *J Cell Mol Med* 2011;**15**:1266–86
49 Wu L, Cai X, Zhang S, Karperien M, Lin Y. Regeneration of articular cartilage by adipose tissue derived mesenchymal stem cells: perspectives from stem cell biology and molecular medicine. *J Cell Physiol* 2013;**228**:938–44
50 Birchall MA, Seifalian AM. Tissue engineering's green shoots of disruptive innovation. *Lancet* 2014;**384**:288–90
51 Fulco I, Miot S, Haug MD, Barbero A, Wixmerten A, Feliciano S *et al*. Engineered autologous cartilage tissue for nasal reconstruction after tumour resection: an observational first-in-human trial. *Lancet* 2014;**384**:337–46
52 Fishman JM, De Coppi P, Elliott MJ, Atala A, Birchall MA, Macchiarini P. Airway tissue engineering. *Expert Opin Biol Ther* 2011;**11**:1623–35
53 Tojo T, Niwaya K, Sawabata N. Tracheal replacement with cryopreserved tracheal allograft: experiment in dogs. *Ann Thorac Surg* 1998;**66**:209–13
54 Macchiarini P, Jungebluth P, Go T, Asnaghi MA, Rees LE, Cogan TA *et al*. Clinical transplantation of a tissue-engineered airway. *Lancet* 2008;**372**:2023–30
55 Gonfiotti A, Jaus MO, Barale D, Baiguera S, Comin C, Lavorini F *et al*. The first tissue-engineered airway transplantation: 5-year follow-up results. *Lancet* 2014;**383**:238–44
56 Fishman JM, Wiles K, Lowdell MW, De Coppi P, Elliott MJ, Atala A *et al*. Airway tissue engineering: an update. *Expert Opin Biol Ther* 2014;**14**:1477–91

57 Fishman J, Lowdell M, Ansari T, Sibbons P, De Coppi P, Birchall M. Characterisation and immunogenicity of a decellularised skeletal muscle scaffold for laryngeal tissue engineering. *Lancet* 2013;**381**:S42
58 Fishman JM, Lowdell MW, Urbani L, Ansari T, Burns AJ, Turmaine M *et al*. Immunomodulatory effect of a decellularized skeletal muscle scaffold in a discordant xenotransplantation model. *Proc Natl Acad Sci USA* 2013;**110**:14360–5
59 Johnson BQ, Fox R, Chen X, Thibeault S. Tissue regeneration of the vocal fold using bone marrow mesenchymal stem cells and synthetic extracellular matrix injections in rats. *Laryngoscope* 2010;**120**:537–45
60 Svensson B, Nagubothu SR, Cedervall J, Chan RW, Le Blanc K, Kimura M *et al*. Injection of human mesenchymal stem cells improves healing of vocal folds after scar excision--a xenograft analysis. *Laryngoscope* 2011;**121**:2185–90
61 Zopf DA, Hollister SJ, Nelson ME, Ohye RG, Green GE. Bioresorbable airway splint created with a three-dimensional printer. *N Engl J Med* 2013;**368**:2043–5
62 Aframian DJ, Palmon A. Current status of the development of an artificial salivary gland. *Tissue Eng Part B Rev* 2008;**14**:187–98
63 Ogawa M, Oshima M, Imamura A, Sekine Y, Ishida K, Yamashita K *et al*. Functional salivary gland regeneration by transplantation of a bioengineered organ germ. *Nature Commun* 2013;**4**:2498
64 Joraku A, Sullivan CA, Yoo J, Atala A. In-vitro reconstitution of three-dimensional human salivary gland tissue structures. *Differentiation* 2007;**75**:318–24
65 Xiong X, Shi X, Chen F. Human adipose tissue-derived stem cells alleviate radiation-induced xerostomia. *Int J Mol Med* 2014;**34**:749–55
66 Jeong J, Baek H, Kim YJ, Choi Y, Lee H, Lee E *et al*. Human salivary gland stem cells ameliorate hyposalivation of radiation-damaged rat salivary glands. *Exp Mol Med* 2013;**45**:e58
67 Lim JY, Ra JC, Shin IS, Jang YH, An HY, Choi JS *et al*. Systemic transplantation of human adipose tissue-derived mesenchymal stem cells for the regeneration of irradiation-induced salivary gland damage. *PloS one* 2013;**8**:e71167
68 Watanabe Y, Sasaki R, Matsumine H, Yamato M, Okano T. Undifferentiated and differentiated adipose-derived stem cells improve nerve regeneration in a rat model of facial nerve defect. *J Tissue Eng Regen Med* 2014. Epub 2014 Jun 1
69 Matsumine H, Sasaki R, Tabata Y, Matsui M, Yamato M, Okano T *et al*. Facial nerve regeneration using basic fibroblast growth factor-impregnated gelatin microspheres in a rat model. *J Tissue Eng Regen Med.* 2014. Epub 2014 Apr 16
70 Cui Y, Lu C, Meng D, Xiao Z, Hou X, Ding W *et al*. Collagen scaffolds modified with CNTF and bFGF promote facial nerve regeneration in minipigs. *Biomaterials* 2014;**35**:7819–27
71 Chen D, Chen S, Wang W, Zhang C, Zheng H. Spontaneous regeneration of recurrent laryngeal nerve following long-term vocal fold paralysis in humans: histologic evidence. *Laryngoscope* 2011;**121**:1035–9
72 Halum SL, McRae B, Bijangi-Vishehsaraei K, Hiatt K. Neurotrophic factor-secreting autologous muscle stem cell therapy for the treatment of laryngeal denervation injury. *Laryngoscope* 2012;**122**:2482–96
73 Lerner MZ, Matsushita T, Lankford KL, Radtke C, Kocsis JD, Young NO. Intravenous mesenchymal stem cell therapy after recurrent laryngeal nerve injury: a preliminary study. *Laryngoscope* 2014;**124**:2555–60
74 Herford AS, Boyne PJ. Reconstruction of mandibular continuity defects with bone morphogenetic protein-2 (rhBMP-2). *J Oral Maxillofac Surg* 2008;**66**:616–24
75 Lee MK, DeConde A, Aghaloo T, Lee M, Tetradis S, St John M. Biomimetic scaffolds loaded with adipose-derived stem cells and BMP-2 induce healing of mandibular defects. *Otolaryngol Head Neck Surg* 2013;**1**:35–6
76 Kelly MP, Savage JW, Bentzen SM, Hsu WK, Ellison SA, Anderson PA. Cancer risk from bone morphogenetic protein exposure in spinal arthrodesis. *J Bone Joint Surg Am* 2014;**96**:1417–22

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