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Running Title

Regenerative Otorhinolaryngology

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Abstract

Objective

Tissue engineering using biocompatible scaffolds, with or without cells, can permit surgeons to restore structure and function following tissue resection or 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.

Review Methods

A systematic review of the literature was performed using Ovid Medline and Ovid Embase from database inception to 15th 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.

Conclusions

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

Key Words

Regenerative surgery; Regenerative medicine; Stem cells; Tissue engineering; Otorhinolaryngology; Otology; Audiological medicine; Laryngology; Rhinology; Head and neck; Reconstruction

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“Regenerative Surgery”, “Audiological Medicine”, “Rhinology”, “Head And Neck” and “Reconstruction” are not MeSH-approved key words. Please can you clarify whether you want to replace, retain or delete the non-MeSH key words?

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, permit surgeons to restore or, in the case of congenital abnormalities, establish normal structure and function in: ~~applications as diverse as replacing the airways (airway replacement)~~, the gastrointestinal tract, hepatobiliary system, myocardium, renal and urinary tract, 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 biologic scaffolds), and appropriate signaling (physical or chemical).^{10,11} Tracheal replacement and regeneration has emerged as a spearhead application of these technologies¹⁰, 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.

Methods

We conducted a review of the literature surrounding regenerative medicine, tissue engineering and their application in ENT surgery. The principle author (JCRW) performed a literature search of Ovid Medline and Ovid Embase using variants of the search terms 'regenerative medicine', 'regenerative surgery', 'stem cells', 'tissue engineering', 'tissue transplantation', 'otology', 'rhinology', 'laryngology', 'head and neck', 'otolaryngology' and

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'otorhinolaryngology from database inception to 15th November 2014. MeSH 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 the relevance to the topic of interest and were excluded if they were not in English or were duplicate in material covered. Review articles were preferred to original papers in topics where there was more substantial literature, whereas primary research articles were preferred in more innovative areas. The references of relevant articles were analysed for further 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 on-going clinical trials (Figure 1).

Discussion

Otology and audiological medicine

A prominent aspect of regenerative medicine of ear disease is the potential use of stem cells to regenerate irreversible hearing loss in sensorineural disease. Sensorineural hearing loss is a result of dysfunction of the inner ear cochlea, involving the loss of sensory hair cells and/or a secondary degeneration of spiral ganglion neurons (SGNs). It is the commonest disability in developed countries with a considerable socio-economic impact.¹¹ Ageing is a major factor in hearing decline,¹² with an estimated 63.1% of individuals of 70 years and older living with hearing loss in the United States.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 increasingly of concern with ageing populations and exposure to loud environments, particularly in the younger demographic.¹⁶ 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: 1. Differentiation of endogenous stem cells into new cochlear hair cells; 2. 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 is some (*in vitro*) data to suggest the presence of a limited number of cells possessing favorable characteristics including limited proliferation capability²⁰ 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; either by de-differentiating them into a more naïve state normally only found during development to regenerate sensory cells; by direct transdifferentiation of the cells to the target cell type of interest; or by 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 (iPSCs), mesenchymal stem cells (MSCs) 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 tumorigenic 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 immunologic environment, the complex architecture of the Organ of Corti, and the repair mechanisms of the cochlea, which results 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 cells²⁴⁻²⁶. However, the research is still in its early stages and translation to the clinical setting is distant, while the above issues are still being addressed.

The generation of new hair cells and auditory neurons is particularly challenging due to the importance of hair cell alignment and tonotopic organization. Regenerated hair cells must be correctly oriented 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 (ESCs), iPSCs and fetal auditory stem cells has been a promising area of research.²⁸ Most recently, Koehler et al created step-wise differentiation of inner ear sensory epithelia from mouse ESCs in three-dimensional culture. Using an *in vivo* three-dimensional culture in combination with bone morphogenetic protein (BMP) activation and transforming growth factor beta (TGF- 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.²⁵ 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 both genetic and molecular therapies for this condition.²⁷

Surgical intervention to treat middle ear diseases can disrupt the middle ear mucosa, ~~an epithelium with that functions in the to maintainance of middle ear pressure~~, 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 ~~treatment potential~~ appears to be the use of transplantation of 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 by culturing MSCs under osteogenic conditions on bioresorbable 3-dimensional scaffolds.^{31,32}

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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 base is in animal models with 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 (TM) perforations to receive basic fibroblast growth factor (b-FGF) on a gelatin sponge with fibrin glue, or gelatin sponge plus fibrin glue alone. The biomolecular intervention significantly improved healing rates and mean hearing level compared to controls.³⁴ 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 randomized, unblinded trial of acellular dermis versus temporalis fascia for tympanoplasty found that acellular dermis provided 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 MSCs to rats with chronic TM 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. To replace this method, which involves multi-stage surgery and permanent loss of cartilage from the donor site, a stem cell source combined with a compatible scaffold to mimic cartilage would be a good alternative.³⁷ Recent work using adipose-derived stem cells (ASCs) 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 directly comparing tissue-engineered with conventional (autologous grafting) techniques. Such techniques may be especially useful in revision cases where autologous graft material may be lacking.

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Rhinology

Unlike in otology and laryngology, rhinology plays a greater role in the provision of stem cells rather than in receipt. The nose contains several populations of autogenous stem cells that are relatively easy to access, 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 (OECs), a form of glial cell with the potential to support neural regeneration within the olfactory epithelium³⁹ and also when transplanted into other tissues such as the brain and spinal cord.⁴⁰⁻⁴² ~~De Corgnol Marie~~ and colleagues investigated the *in vivo* use of OECs in rats to assist surgical reinnervation of the vocal cords following vagus nerve section. Rats with the OEC intervention had improved reinnervation results compared to controls in terms of post-operative vocal cord function.⁴³

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Choi et al identified the nasal septal mucosa, particularly the superior-posterior areas, as a rich source of OECs by prospectively analyzing nasal biopsies, taken during transphenoidal surgery.⁴⁴ There has also been some suggestion of the existence of a MSC population in the olfactory epithelium^{45,46} as well as the respiratory epithelium⁴⁷. 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 an observational first-in-human clinical trial, five patients recently received tissue-engineered cartilage implants for nasal reconstructive surgery.^{50,51} The investigators report 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 for transplant but both convey considerable morbidity, including foreign body reactions and fibrotic stenosis. Fresh, viable tracheal allografts usually require considerable immunosuppressive therapy and 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 utilized in a patient. 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 MSC-derived chondrocytes.⁵⁴ 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 has recently been reported, indicating positive long-term outcomes⁵⁵. 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. By using a decellularised cadaveric trachea seeded with autologous MSCs and exposed to topical biochemical inducers of differentiation, the airway was successfully reconstructed with total functionality at 2-years post-op.¹⁰

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These significant advances in generation of stem cell-derived airway grafts and the development of advanced bioreactors has lead 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. They found that their decellularization process resulted in total DNA clearance and down-regulation of **major histocompatibility complex** ~~MHC~~ expression whilst maintaining the scaffold's structural integrity. This improved the longevity of the xenogenic bioscaffold by reducing the cell-mediated immune response, also increasing 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 the vocal cords 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 also a promising area of research. A small animal study (n=12) using injectable MSCs improving vocal cord healing in rabbits found better viscoelasticity of the cords and enhanced healing following injury.⁶⁰

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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 airways 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 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, 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 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 three-dimensional tissue engineering methods *in vitro*.⁶⁴ However, at time of writing, this has not been translated into clinical use. In contrast, the use of direct cellular therapy has appeared to have more promising results. Xiong and colleagues exposed rats to radiotherapy to replicate this pathology then injected human ASCs into their submandibular salivary glands. The intervention improved salivary gland flow rate and post-radiotherapy healing compared to control.⁶⁵ 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 iPSCs, MSCs and Schwann cells for regeneration of the facial nerve. Research in this field is limited to the animal model currently. Watanabe et al utilised ASCs, retrieved during liposuction to regenerate a 7mm gap in rat facial nerve and compared this technique directly with autologous grafting. Some of the ASCs were

differentiated into Schwann-like cells and some remained undifferentiated. The ASCs were then embedded onto a collagen scaffold and implanted into the facial nerve defect. Functional recovery was similar between both groups and both differentiated and undifferentiated ASCs were capable of neuroregeneration.⁶⁸ Basic fibroblast growth factor (bFGF) promotes regeneration of peripheral nerve defects by affecting nerve cells, Schwann cells and fibroblasts, and increasing rate of nerve regeneration, number of regenerated nerves and degree of maturation of regenerated nerves.⁶⁹ Cui et al utilised a functional nerve conduit consisting of a collagen scaffold plus neurocytokines ciliary-derived neurotropic factor (CNTF) and bFGF to repair a considerably larger facial nerve gap of 35mm in a mini-pig model. 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 6 months post-implantation, they found that the combination of CNTF and bFGF promoted facial nerve regeneration (rather than single neurocytokines) in terms of 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 CNTF-secreting MSCs to attempt to promote this phenomenon. Immunohistochemical analysis identified that the MSCs in combination with **nuclear factor NF** selectively enhanced reinnervation motor neuron regeneration, although the functional outcome on laryngeal muscle was unclear from this study.⁷² The use of intravenous stem cell therapy, usually MSCs has shown promise for the regeneration of neuronal tissue following stroke and spinal cord injury. Lerner et al performed a pilot randomised trial **of the** use of **intravenous IV**-MSCs to assess recovery of the RLN following injury in 12 rats. They found no significant difference between the MSC group compared to control, although the data were promising for enhanced functional recovery in the experimental arm.⁷³

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

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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-signaling factors to stimulate new bone growth *de novo* are currently under investigation. A small case series (n=14) of patients underwent mandibular reconstruction with bone morphogenetic protein-2 (BMP-2), a bone-promoting cytokine, in a collagen matrix carrier as an alternative to autologous bone graft. All 14 patients developed new bone tissue at the graft site, palpable by 3 to 4 months and radiographically evident at 6 months.⁷⁴ This 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 and then infiltrated with rat ASCs and/or BMP-2. These grafts were then implanted into 28 rats with large mandibular defects. The authors found that the bioscaffold produced bone regeneration with ASCs, BMP-2 infiltration and both in combination.⁷⁵ There are concerns regarding the risk of malignancy following the use of BMP, as with many cellular therapies, although interestingly a large retrospective series of spinal surgery patients in the US found no increased risk of cancer following BMP use, contradicting previous findings.⁷⁶

Conclusions

Regenerative approaches can be readily applied to diseases of the ear, nose and throat (ENT), especially where conventional interventions are limited. Each sub-specialty 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 reconstruction of the head and neck, as well as 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, there is currently little known about the potential for stem cell therapy in diseases of the vestibular system, such as Meniere's disease and vestibular neuritis.

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

Summary

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- Regenerative approaches can be readily applied to diseases of the ear, nose and throat (ENT), especially where conventional interventions are limited.
- Each sub-specialty of ENT is developing novel regenerative interventions based on the principles of tissue engineering and cellular therapy.
- There is on-going work on the use of stem cells in reconstruction of the head and neck, as well as a variety of related surgical specialties.
- Despite the advancements in the use of stem cell therapy to restore hearing loss secondary to inner ear disease, there is currently little known about the potential for stem cell therapy in diseases of the vestibular system, such as Meniere's disease and vestibular neuritis.
- The main issues currently facing research in regenerative surgery are the translation of animal model work into human models, increasing the availability of stem cells that can be used to further research and the development of better facilities to enable better implementation of these advances.
- Otorhinolaryngology provides some of the most complex challenges for regenerative medicine: attaining stem cells from the inner ear for further research and reconstructing the complex anatomy of the larynx using vocal fold scaffolds, among others.

Figure 1

Article Attrition Chart

*MeSH terms: otolaryngology, nose diseases, otorhinolaryngologic diseases, larynx, head, neck, head and neck neoplasms, regenerative medicine, tissue engineering, tissue transplantation, stem cells

**Search refined with Boolean operators : AND, OR

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