Transplantation of Mesenchymal Stem Cells

Subjects: Otorhinolaryngology

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Otorhinolaryngology enrolls head and neck surgery in various tissues such as ear, nose, and throat (ENT) that govern different activities such as hearing, breathing, smelling, production of vocal sounds, the balance, deglutition, facial animation, air filtration and humidification, and articulation during speech, while absence of these functions can lead to high morbidity and even mortality. Conventional therapies for head and neck damaged tissues include grafts, transplants, and artificial materials, but grafts have limited availability and cause morbidity in the donor site. To improve these limitations, regenerative medicine, as a novel and rapidly growing field, has opened a new therapeutic window in otorhinolaryngology by using cell transplantation to target the healing and replacement of injured tissues. There is a high risk of rejection and tumor formation for transplantation of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs); mesenchymal stem cells (MSCs) lack these drawbacks. They have easy expansion and antiapoptotic properties with a wide range of healing and aesthetic functions that make them a novel candidate in otorhinolaryngology for craniofacial defects and diseases and hold immense promise for bone tissue healing; even the tissue sources and types of MSCs, the method of cell introduction and their preparation quality can influence the final outcome in the injured tissue.

Keywords: mesenchymal stem cells ; transplantation ; otorhinolaryngology ; head and neck surgery

1. Introduction

Head and neck structures are responsible for vital activities of swallowing and breathing and facilitate our sense of self by vocal communication, physical appearance, facial animation, and hearing, while lack of these activities can influence the quality of life and result in loss of life. In head and neck diseases and disorders, patients are expected to refer to an otorhinolaryngologist to search for treatment of damaged tissues in head and neck structures because the otorhinolaryngology field enrolls head and neck surgery in various tissues such as ear, nose and throat (ENT) that govern different activities such as hearing, breathing, smelling, production of vocal sounds, the balance, deglutition, facial animation, air filtration and humidification, and articulation during speech. Therefore, absence of these functions can lead to high morbidity and even mortality [1][2].

Conventional therapies for head and neck damaged tissues include artificial materials and grafts from other tissues [2], but grafts were shown to have limited availability and can lead to morbidity in the donor site [3], and the use of artificial materials can have the risk of infection and reaction by the immune system [1]. When grafts are undertaken, immunosuppressive drugs are needed that have limited availability in many regions [4]. To improve these limitations, regenerative medicine, by using cell transplantation, has opened a new therapeutic window, which is a novel and rapidly growing field in otorhinolaryngology, which targets the healing and replacement of injured tissues where no current standard therapy works to restore functions of otorhinolaryngology sites [5]. In this review, we described achievements and challenges in regenerative medicine research using cell transplantation in otorhinolaryngology and head and neck surgery fields.

2. Sources and Selection Criteria

Articles published in PubMed and Scholar Google from 2003 to 2021 were searched using search terms: “stem cell”, “cell transplantation”, “regenerative medicine”, “scaffold” and “tissue engineering” with “ear”, “hearing”, “tympanic membrane”, “cochlea”, “nose”, “vocal fold”, “larynx”, “sinus”, “craniofacial”, and “head and neck”.

3. In Vivo Studies of Inner Ear Hearing Loss

Table 1 presents in vivo studies of inner ear hearing loss based on stem cell source and year of study. Several types of stem cells, including BMSCs, AdSCs, umbilical cord stem cells (UCSCs), tongue-derived stem cells (TSCs), olfactory epithelium neural stem cells (oeMSCs), nasal tissue-derived stem cells (NSCs), and hematopoietic stem cells (HSCs)

have been utilized in the treatment of inner ear hearing loss in animal models of mouse, rat, gerbil, and Guinea pig. There are many studies regarding the use of BMSCs in treatment of inner ear hearing loss. Li et al. used BMSCs in rats and observed cell transformation into neuron-like cells and positive expression of neurofilament (NF-200), microassociate protein-2 (MAP-2), neuron-specific nuclear protein (NeuN), nestin, gial fibrillary acidic protein (GFAP), GAD, and ChAT by immunohisitochemistry [6]. Intravenous transplantation of BMSCs and HSCs in mice resulted in integration of cells in the cochlea, and their differentiation, to inner ear fibrocyte-like cells without any adverse effects on auditory function [7]. Perilymphatic transplantation of green fluorescent protein (GFP) transgenic mouse BMSCs in the gerbil model of auditory neuropathy (deafened with ouabain) via scala tympani or modiolar injection demonstrated survival of MSCs within the modiolus that participated in the regeneration of damaged SGNs without any evidence of hyperacute rejection [8]. Transplantation of BMSCs into lateral semicircular canals in the rat model of acute SNHL, secondary to fibrocyte dysfunction (mitochondrial toxin), resulted in detection of MSCs in injured lateral cochlear wall, expression of connexin 26 and connexin 30, reactivation in gap junction between neighboring cells, an increase in cell survival, and acceleration of hearing recovery through the repair of injured cochlear fibrocytes [9]. Ratajczac et al. utilized BMSCs in mice and noted very small, embryonic-like cells with the potential to develop into neural and other cells for tissue repair [10]. GFP transgenic mouse BMSCs transplantation into the perilymphatic space of normal cochleae in mice displayed that transplanted cells could settle within the cochlear tissues, especially in the SLs and the spiral limbus, although most transplants were located in the perilymphatic space. Some of the transplanted cells expressed the cochlear gap-junction protein connexin 26, indicating their potential for restoration of cochlear cells [11].

Table 1. In vivo studies in treatment of inner ear hearing loss.

Type of Stem Cell	Animal Model	Hearing Loss Model	Outcome	Reference
BMSCs and HSCs (EGFP labeled)	Mouse	Irradiated deafened by a single 950-cGy dose of total body	Integration of cells in the cochlea and differentiation to inner ear SLF without any adverse effects on +auditory function	[7]
BMSCs	Gerbil	Auditory neuropathy deafened with ouabain	Survival of MSCs within the modiolus, regeneration of damaged SGNs without any evidence of hyperacute rejection	[8]
BMSCs	Rat	Acute SNHL secondary to fibrocyte dysfunction [mitochondrial toxin]	Expression of connexin 26 and connexin 30, reactivation in gap junction between neighboring cells, and acceleration of hearing recovery	[9]
BMSCs (GFP-labeled)	Mouse	Normal cochleae	Settled cells within the cochlea, expression of cochlear gap-junction protein connexin 26, and restoration of cochlear cells	[11]
BMSCs	Guinea pig	Autoimmune deafened adult	Homing and survival capability of cells in cochlea, and transdifferentiation of MSCs to cochlea cell types	[12]
BMSCs	Guinea pig	Ouabain-induced auditory neuropathy	An increase in SGN number, and improvement of hearing function	[13]
BMSCs	Rat	Noise-induced or ototoxic SNHL	Survival of a small number of MSCs within the spiral ganglion area	[14]
BMSCs and AdSCs	Guinea pig	Deafened model	Differentiation into neuron-like cells	[15]
BMSCs	Mouse	Damaged SLF network	Functional hearing recovery after cell transplantation	[16]
BMSCs (EGFP)	Mouse	SNHL	Cell migration to cochlea and differentiation into SLF in absence of adverse effects on auditory brainstem response	[17]
BMSCs	Guinea pig	Neomycin-deafened	An increase in number of SGNs in organ of Corti and spiral ganglion and differentiation into neuronal progenitor cells and neuronal cells, treatment of SNHL	[18]
BMSCs (Magnetic labeled)	Guinea pig	Cochleostomy deafened	Successful engraftment in the inner ear	[19]
BMSCs	Mammalian	Structural reorganization of the damaged cochlea	improve incomplete hearing recovery	[20]

Type of Stem Cell	Animal Model	Hearing Loss Model	Outcome	Reference
BMSCs	Mouse	Degeneration of cochlear fibrocytes in the spiral ligament [SL] using local application of 3-nitropropionic acid [3-NP]	Regeneration and maintenance of fibrocytes in damaged spiral ligaments, partial restoration of cochlear function	[21]
BMSCs	Rat	Cochlear insult	No recruitment of inflammatory leukocytes and edema in the cochlea	[22]
AdSCs	Guinea pig	Noise deafened adult	Cell survival and migration at the site of tissue damage, and treatment of deafness	[23]
AdSCs	Mouse	Autoimmune hearing loss model by β-tubulin	Immunomodulatory properties, absence of atrophy in stria vascularis or organ of Corti, and improvement in hearing function	[24]
AdSCs	Rat	Kanamycin deafened	Elevations of BDNF, significant number of MSCs in the cochlea, improved survival of SGNs, and improved hearing threshold levels	[25]
UCSCs	Guinea pig	SNHL deafened by neomycin and ouabain octahydrate into middle ear	A rise in the number of SGNs, improvement in hearing thresholds	[26]
TSCs	Mouse	Noise deafened	Attenuating ototoxic effects of noise trauma	[27]
oeMSCs	Mouse	Lateral wall cochleostomy hearing loss	Survival of implanted stem cells, transdifferentiation into SLF and conservation of hearing	[28]
oeMSCs	Rat	Noise-induced hearing loss	Cell migration around the SGN and restoration of hearing loss	[29]
oeMSCs	Mouse	Lateral wall cochleostomy with early onset progressive SNHL	A reduction in inflammation, oxidative stress and cell apoptosis, significant lower hearing threshold levels and amelioration of hearing loss	[30]
NSCs	Rat	Spiral ganglion loss	Neuronal differentiation, repair in injured cochlea	[31]

Tan et al. in autoimmune deafened adult Guinea pig by use of IL-4-expressing BMSCs into scala tympani, via lateral wall, reported homing and survival capability of cells to the deafened cochlea, and transdifferentiation of them to any cochlea cell types [12]. Cho et al. utilized human BMSCs in vitro neural differentiated cells into the scala tympani to treat a Guinea pig animal model with ouabain-induced auditory neuropathy, leading to an increase in SGNs number and improvement of hearing function [13]. Intravenous injection of human BMSCs in a rat model of noise-induced or ototoxic SNHL resulted in survival of a small number of MSCs within the spiral ganglion area while most MSCs were trapped in the lungs [14]. In the Guinea pig model, the potential of AdSCs and BMSCs differentiation into neuron-like cells was demonstrated [15]. In mice, hearing recovery, after transplantation of BMSCs into the inner ear, was shown to happen, due to transdifferentiation of BMSCs into sensory fibrocyte-like (SFL) cells and stimulation of host SFLs regeneration [16].

Mouse MSCs transplanted into ampulla of superior semicircular canal in young and old healthy mice could show migration of BMSCs to cochlea, and their differentiation into SFL cells, in young mice without any adverse effects on auditory brainstem response (ABR) [17]. Human BMSCs transplanted into neomycin-deafened Guinea pig cochlea led to an increase in number of SGNs in organ of Corti and spiral ganglion, and their differentiation into neuronal progenitor cells and neuronal cells, to be used in treatment of SNHL, based on the induction of stem cell homing factors in the host cochlear tissue [18]. Magnetic labeled MSCs have been investigated in otorhinolaryngology too. To track the condition of cells transplanted in the inner ear, superparamagnetic iron oxide nanoparticles (SPIONs) have been incorporated into BMSCs, and the cells were injected to the inner ear of Guinea pig and monitored by a 1.5 Tesla MRI (Siemens, Munich, Germany) to confirm that the stem cells were successfully engrafted in the inner ear [19]. Mahmoudian-Sani et al. suggested that BMSCs, in comparison to AdSCs and UCSCs, had more efficacy to migrate and survive in the cochlear tissues, regenerating inner ear, and treating SNHL [20].

In mice, BMSCs were shown to enhance the regeneration and maintenance of fibrocytes in damaged SLs, leading to partial restoration of cochlear function [21]. Transtympanic transplantation of rodent BMSCs in a non-immunocompromised rat model to assess cochlear function by ABR, distortion product otoacoustic emissions (DPOAE), and histopathology did not reveal the recruitment of inflammatory leukocytes and edema in the cochlea of MSCs administered rats [22]. Fetoni et

al., in a noise deafened adult Guinea pig, found that transplanted AdSCs into scala tympani, via round window, were able to survive and migrate, at the site of tissue damage, and express trophic factors to pave the way for further treatment of deafness [23]. Intraperitoneal injection of human AdSCs, two weeks after the onset of hearing loss, in mouse model of experimental autoimmune hearing loss (EAHL) was treated with β-tubulin, demonstrated immunomodulatory properties, absence of atrophy in stria vascularis or organ of Corti, and improvement in hearing function, presented as decreased thresholds of ABR, and protected HCs in established EAHL [24].

Injection of magnetically labeled AdSCs into the cochlea of kanamycin deafened rats revealed elevations of BDNF, the presence of significant number of MSCs in the cochlea, improved survival of SGNs, and improved hearing threshold levels denoting to their protective effects against loss of auditory function [25]. Intravenous transplantation of intact human UCSCs in a deaf Guinea pig model revealed an improvement in hearing thresholds via relocation and a rise in the number of SGNs [26]. Sullivan et al., in an adult mouse deafened by noise, demonstrated that administration of mouse TSCs into scala tympani/scala vestibulivia lateral wall led to an increase in cell survival and could attenuate the ototoxic effects of noise trauma [27]. The administration of oeMSCs into the mice cochleae, with lateral wall cochleostomy hearing loss, exhibited survival of implanted stem cells within the perilymphatic spaces of the scala tympani and conservation of hearing with otoprotective activity of oeMSCs, via stimulation by native spiral ligament fibrocytes and transdifferentiation into SFLs and stimulation of regeneration in situ of host spiral ligament fibrocytes, from a resident stem cell population by paracrine nature of MSCs [28].

Direct injection of rat oeMSCs, into the cochlear of noise-induced hearing loss model of rats, resulted in migration of stem cells around the spiral ganglion neurons and restoration of hearing loss after cell implantation, as assessed by ABR [29]. Injection of human olfactory stem cells (OSCs) into cochleae of mice, via lateral wall cochleostomy, with early onset progressive SNHL resulted in a reduction in inflammation, oxidative stress, cell apoptosis, a significant lower hearing threshold level, and amelioration of hearing loss [30]. The human MSCs, derived from nasal tissue, were evidenced to repair spiral ganglion loss in experimentally injured cochlear of neonatal rats via direct neuronal differentiation and secondary effects on endogenous cells [31] that can be tracked and verified, in a non-invasive manner, after cell transplantation by using MRI contrast agents [19][25][32].

4. Tympanic Membrane Hearing Loss

Chronic otitis media is the primary cause of conductive hearing loss that involves perforation of the tympanic membrane (TM) and erosion of the ossicles. The TM, or eardrum, is a thin, protective layer of middle-ear tissue that forms a boundary between the external and middle ear. It is consisted of three main parts of larger pars tensa, the smaller pars flaccida, and umbo that are also other components of the hearing process in the auditory system. The anatomical structure of the TM has three layers of ectoderm, mesoderm, and endoderm [33].

TM is responsible for amplifying and transmission of sound vibrations through a chain of mobile ossicles and its perforations. External sound pressure, middle ear infection, severe trauma, and insertion of sharp objects into the ear can lead to deficient hearing function. Three primary issues in the repair of TM perforation have been reported including absence of structural assistance, absence of extracellular matrix, leading to weak neomembrane adhesion of cells, and limited angiogenesis and growth factors [34]. It is necessary to mention that wound healing in TM is slightly different from wound healing in other cutaneous tissues [35], so an exudate is released around the edges of the perforated TM, after injury, which can protect the tissue from dehydration and facilitate cell migration and proliferation of stratified squamous epithelial layer to the perforation center [36].

To close the perforated eardrum, surgical procedures (myringoplasty or tympanoplasty) are undertaken by otorhinolaryngologists, but limitations, such as discomfort, side effects, and high cost of surgical treatment have necessitated the use of better alternatives, such as tissue engineering and MSCs transplantation, as a promising tool to overcome the limitations, the operational risks and to restore, to maintain, and to improve the TM function [33]. In an injured eardrum, cell-based therapies have opened a way to solve these limitations, because MSCs can migrate towards the site of injury and participate in cell survival, cell proliferation, and tissue angiogenesis by secretion of trophic factors such as vascular endothelial growth factor (VEGF), EGF, IGF, hepatocyte growth factor (HGF), nerve growth factor (NGF), TGF-α, and stromal derived factor-1 (SDF-1), along with chemoattractant gradients in the stromal extracellular matrix and peripheral blood [37], where local factors such as hypoxia, toll-like receptor ligands, and the cytokines activate the MSCs to foster the entry of more growth factors to boost tissue regeneration [38].

In this relation, MSCs in the TM must have an appropriate microenvironment to facilitate cell survival and proliferation. If, during introduction of MSCs in the perforated TM, the cells are dropped into the middle ear cavity, the cells would be

easily susceptible to air-drying through external auditory meatus [39]. Another important point in cell transplantation is the cell delivery that uses of scaffolds as an increasingly popular technique that can provide protection and controlled spatial cues for seeded stem cells. The delivery of MSCs at the ruptured TM sites was shown to enhance the activation of epithelial stem cells for faster closure of TM perforation. So, the fibroblasts and collagen in the middle connective tissue layer produce a neomembrane framework to close the perforation [40]. Danti et al. have fabricated colonization of human MSCs on scaffolds to allow an osteoblastic maturation in vitro [41].

5. In Vivo Studies of Tympanic Membrane Related Hearing Loss

Rahman et al. used drops of gelatin, containing human BMSCs, on the perforated TM of rats and assessed the thickness of pars tensa region and lamina propria under otomicroscopy, mechanical stiffness of the healed TM tissue by Moiré interferometry and lamina propria, middle ear cavity, and external ear canal wall tissue via microscopy and illustrated a decrease in the stiffness of the healed tympanic membrane and a healing process with an enhanced restoration [42]. When GFP expressing BMSCs were embedded in porcine-derived (Gelita-Spon GS), hyaluronate-derived (EpiDisc ED), and polyvinyl alcohol (PVA) scaffolds and injected in an injured TM of a mouse model, the transplanted cells were deposited in the injured tissue and differentiated into epithelial-like cells and formed a thicker neotympanum that can be a promising alternative to tympanoplasty [43].

The first animal model trial of concurrent use of MSCs and a three-dimensional (3D) bioprinted scaffold (polycaprolactone/collagen/alginate) was carried out in closing of subacute TM perforations in rats undergoing otoendoscopy for acoustic measurements as per ABR thresholds. The findings denoted to the recovery of the hearing capacity at all frequencies, along with regeneration of the thick neodrum assessed by optical coherence tomography (OCT). Goncalves et al., by use of BMSCs seeded on hyaluronic acid (HA) scaffold in mice bilateral large tympanic perforations, demonstrated the repair in TM and restoration of the trilaminar structure in TM. Assessment by histology indicated formation of an intact neotympanum in the perforated areas. Neo-tympanal integrity and transparency were confirmed by otoscopy too [44]. The mechanical properties of the regenerated TM, such as membrane stiffness, membrane stability, and efficient nanovibration were evaluated by a laser Doppler vibrometer (LDV), revealing an acceleration in the healing process in the TM perforations and formation of a thickened prominent fibrous layer. The acoustic mechanical properties were recovered in the healed TM [45]. Ong et al. found that human AdSCs in mice with sub-total pars tensa perforations could lead to paracrine function, secretion of growth factors, a promoted significant keratinocyte proliferation and migration and TM wound healing [46]. **Table 2** presents in vivo studies oftympanic membrane related hearing loss based on stem cell source and year of study.

Table 2. In vivo studies of tympanic membrane related hearing loss.

Type of Stem Cell	Animal Model	Tympanic Hearing Loss Model	Outcome	Reference
BMSCs	Rat	Perforated TM	A decrease in the stiffness of the healed tympanic membrane and healing process with an enhanced restoration	[42]
BMSCs, GFP-labeled embedded in porcine GS, hyaluronate-derived ED and PVA	Mouse	Injured TM	Transplanted cells were deposited in the injured tissue, differentiated into SFL, formation of a thicker neotympanum	[43]
BMSCs seeded on HA	Mouse	Bilateral large tympanic perforations	Goncalves et al. by use of Formation of an intact neotympanum, repair and restoration of trilaminar structure in TM, and neo-tympanal integrity and transparency	[44]
BMSCs with a 3D bioprinted scaffold [polycaprolactone/collagen/alginate]	Rat	Subacute TM perforations	Recovery of the hearing capacity at all frequencies, regeneration of the thick neodrum with membrane stiffness and stability, an acceleration in TM healing process	[45]
AdSCs	Mouse	Sub-total pars tensa perforations	Secretion of growth factors, a promoted significant keratinocyte proliferation and migration and TM wound healing	[46]

6. Larynx and Vocal Cord: Larynx

The larynx is a dynamic organ with considerable complexity that should be considered when laryngeal reconstruction is targeted, as a neo-larynx needs functional muscle tissue with appropriate re-innervation. Therefore, decellularized skeletal muscle matrices are utilized as a potential scaffold for production of the muscular activity required for an engineered larynx [47]. Impairment in laryngeal function related to vocalization, swallowing, and respiration can be life challenging and devastating. Problems with swallowing, taste, speech, smelling, breathing, lifting, and aesthetic appearance can lead to a substantial impairment of quality of life, and can affect social functioning and the ability to work. To treat stenotic airway, especially in the subglottic area of laryngotracheal defected patients, laryngotracheoplasty is undertaken, which involves the use of cartilage interpositional grafting. Although a total laryngeal transplantation and replacement would significantly improve the quality of life for these patients, but problems associated with this procedure requiring life-long immunosuppression represent a major ethical question and limitation for the procedure [48].

Among laryngotracheal defects, laryngotracheal stenosis is the most often encountered case with considerable morbidity and mortality that happens congenitally or acquired after prolonged intubation and hypertrophic scarring, and is associated with narrowing of the airway at larynx, subglottis, or trachea [49]. Current choices in treatment of the stenosis are laser surgery, endoscopic dilation, laryngotracheal reconstruction, or life-long tracheostomy, but they can result in formation of new scar tissues and a further restenosis [49]. The regenerative medicine approach, by using MSCs and scaffolds, can represent a significant advantage over these limitations in otorhinolaryngology clinical practices [48].

Transplantation of MSCs was shown to be effective in regeneration of a functional laryngeal tissue and help restoration to a normal anatomy, especially when bioengineering is added to cell therapy that can provide a larger surface area to promote tissue regeneration and increase tissue function [50]. Significant advances have been observed in the generation of stem cell derived airway grafts, construction of a tissue-engineered larynx, and in laryngotracheal stenosis [51] because cell transplantation has anti-inflammatory and immunosuppressive properties, possesses the ability for cell migration to the exact area of injury, and has the potential to secrete soluble factors that are vital for cell survival and proliferation. Cell-based therapies were shown to have minimal side effects and are easily accessible for isolation too [52]. A seeding density exceeding $1 \times 10^6/cm^2$ was illustrated to be an appropriate number of transplanted cells to accelerate the tissue integration process and activate local progenitor cells [53].

7. In Vivo Studies of Larynx

Jotz et al. compared laryngeal defect closure in a porcine model using a naïve nanofiber scaffold seeded with dental pulp stem cells (DPSCs) displaying a significant advantage with formation of neocartilage tissue [54]. Ansari et al. indicated that implantation of a de-cellularized hemi-larynx seeded with human BMSCs in pig models could allow for vascularization and further orthotopic implantation without any adverse effect on respiratory function, swallowing, or vocalization. Rudimentary vocal folds, covered by contiguous epithelium, were also identified [55]. Iravani et al. used BMSCs in laryngotracheal stenosis in a dog model and found a complete epithelialization with minimal chronic inflammatory cell infiltration in the submucosa of vocal folds [56]. Herrmann et al., in implantation of a tissue engineered BMSC in a pig model, found an appropriate mucosal coverage and rudimentary vocal fold development, without any adverse effect on respiratory function, swallowing, or vocalization [48]. **Table 3** presents in vivo studies of laryngeal defects and disorders based on stem cell source and year of study.

Table 3. In vivo studies of laryngeal defects and disorders.

Type of Stem Cell	Animal Model	Defect Model	Outcome	Reference
DPSCs seeded on naïve nanofiber scaffold	Pig	Laryngeal defect	Formation of neocartilage tissue	[54]
BMSCs seeded on de-cellularised hemi-larynx	Pig	Full-thickness defect created in the cricoid cartilage	Vascularization and orthotopic implantation without adverse effects on respiration, swallowing or vocalization and formation of contiguous epithelium and a rudimentary vocal folds	[55]
BMSCs	Dog	Laryngotracheal stenosis	Complete epithelialization with minimal chronic inflammatory cell infiltration in submucosa of vocal folds	[56]

Type of Stem Cell	Animal Model	Defect Model	Outcome	Reference
BMSC seeded on Porcine hemi-larynx de-cellularized	Pig	Defective thyroid cartilage	No adverse effect on respiratory function, swallowing and vocalization, and complete epithelialization of the mucosal surface and the development of rudimentary vocal folds	[48]

8. Larynx and Vocal Fold: Vocal Fold

Based on Hirano's body-cover theory, the vocal folds are consisted of a superior layer ("cover") including epithelium, basal membrane, and the superior part of the lamina propria and an inferior layer ("body") with deep lamina propria and thyroarytenoid muscle being, separated by an intermediate layer of lamina propria. This architecture causes these two functional units to vibrate independently and is found in the mid-part of the vocal folds; the anterior and posterior areas, which are the site of maculae flavae, illustrate a different architecture, which functions as a buffer [57]. After laryngeal microsurgery, vocal fold microstructure and scarring can happen, due to partial disappearance of the lamina propria, with the superficial and/or intermediate layer changed by fibrous tissue, inhibiting mechanical uncoupling of the epithelium and muscle and thereby inducing vibration disorder and disabling dysphonia [58].

Treatment choices, presently, are few, and mostly without efficacy for vibration, posing just an effect on volume to decrease glottal closure defect. So, in the current state of the literature, cell transplantation has been introduced in vocal fold scarring [59]. Chen and Thibeault, in an in vitro study, co-cultured healthy and scarred vocal fold fibroblasts, with BMSCs added to HA hydrogel, and reported an inhibition of fibroblast proliferation without any effect on morphology or viability [60]. Hiwatashi et al., in an in vitro study, investigated the effect of TGF-1 expression by co-culturing normal vocal fold fibroblasts with AdSCs or BMSCs in presence or absence of TGF-1 and found that MSCs could regulate extracellular matrix composition by a decrease in type I and III collagens, inhibited TGF-1 expression, and differentiation toward myofibroblasts, by a decrease in smooth muscle alpha-actin (α-SMA) levels [61]. Kumai et al., in two in vitro studies using ferret AdSCs, showed that when fibroblasts, co-cultured with AdSCs, proliferated less and expressed less α-SMA, less collagen, and more HA and HGF [62][63].

9. Clinical Trials of Vocal Fold Scarring

Karolinska University Hospital, in phase I clinical trial in an open labeled single-group of sixteen 18 years and older patients with severe hoarseness and vocal fold scarring evaluated the injection of autologous BMSCs with a hyaluronan gel. The safety, efficacy, healing process including inflammation, polyp/granuloma formation, and vascularization were assessed. Functional measures, including high-speed imaging, acoustic voice analysis, and phonation pressure measurement were evaluated. The outcome was improved healing of scarred vocal fold, one year postoperatively [64]. Assistance Publique Hopitaux De Marseille in an open labeled single-group clinical trial enrolling eight 18 years and older patients with vocal fold scarring and dysphonia injected autologous AdSCs and reported the feasibility, safety, and efficacy of the procedure and functional measures of voice handicap index evaluated by laryngostroboscopy [65]. Lo Cicero et al. confirmed use of AdSCs in patients who had undergone vocal fold lipoinjection with laryngeal hemiplegia or defects and demonstrated the therapeutic efficacy of this clinical approach and restoration of glottic competence [66]. **Table 4** represents clinical trials in treatment of vocal fold scarring using MSCs based on stem cell source and year of study.

Table 4. Undertaken clinical trials in treatment of vocal fold scarring using MSCs.

Type of Study	No. of Patients	Stem Cell Source (*n*)	Outcome	Reference
Clinical trial phase I	Sixteen 18 years and older with severe hoarseness and vocal fold scarring	BMSCs with a hyaluronan gel	Feasibility, safety, and efficacy of the procedure and functional measures, improved healing of scarred vocal cord one year postoperatively	[64]
Clinical trial	Eight 18 years and older with vocal fold scarring and dysphonia	AdSCs	Positive therapeutic effect of cell transplantation, improved healing of scarred vocal cord	[65]
Clinical trial	12 patients aged 16–66 years with laryngeal hemiplegia or defects	AdSCs	Therapeutic efficacy of cell transplantation in restoration of glottic competence	[66]

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