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REVIEW ARTICLE

Facial Nerve Repair: Bioengineering Approaches in Preclinical Models

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Injury to the facial nerve can occur after different etiologies and range from simple transection of the branches to varying degrees of segmental loss. Management depends on the extent of injury and options include primary repair for simple transections and using autografts, allografts, or conduits for larger gaps. Tissue engineering plays an important role to create artificial materials that are able to mimic the nerve itself without extra morbidity in the patients. The use of neurotrophic factors or stem cells inside the conduits or around the repair site is being increasingly studied to enhance neural recovery to a greater extent. Preclinical studies remain the hallmark for development of these novel approaches and translation into clinical practice. This review will focus on preclinical models of repair after facial nerve injury to help researchers establish an appropriate model to quantify recovery and analyze functional outcomes. Different bioengineered materials, including conduits and nerve grafts, will be discussed based on the experimental animals that were used and the defects introduced. Future directions to extend the applications of processed nerve allografts, bioengineered conduits, and cues inside the conduits to induce neural recovery after facial nerve injury will be highlighted.

Keywords: facial nerve repair, preclinical models, allografts, conduits, stem cells, growth factors

Impact Statement

Recovery after facial nerve injury is a complex process, which involves different management options such as primary repair or the use of nerve grafts or conduits. Various tissue-engineered approaches are increasingly studied on preclinical models with limited, but promising, translation to the clinical setting. Herein, preclinical models focusing on different recovery methods after facial nerve injury are comprehensively reviewed based on the experimental animals used. The review provides key insights into current developments and future directions on this highly relevant topic to help researchers further expand the field of tissue engineering and facial nerve recovery.

Introduction

Facial nerve (the seventh cranial nerve VII; CN VII) is associated with motor, sensory, and parasympathetic functions. After exiting the brain stem, the nerve passes through the internal auditory meatus to enter the temporal bone. The nerve gives greater petrosal (parasympathetic), stapedius (branchial motor), and chorda tympani (parasympathetic and taste) branches in the intratemporal portion and exits the temporal bone through the stylomastoid foramen. The nerve branches into the posterior auricular, posterior belly of digastric, and stylohyoid branches from the trunk before entering the parotid gland. The nerve proceeds to form five main motor branches as temporal, zygomatic, buccal, marginal mandibular, and cervical (Fig. 1).[1,2] These branches are responsible from the innervation of numerous muscles that work together for the complex functions of facial expressions.

Etiologies

Injury to the facial nerve and the resulting facial nerve palsy lead to devastating functional, psychological, and cosmetic challenges.[3,4] There are various etiologies of facial nerve palsy, which can be categorized as idiopathic facial paralysis (Bell's palsy), infectious, neoplastic, developmental, metabolic, toxic, traumatic, and iatrogenic.[2,5,6] Iatrogenic facial nerve injury occurs most commonly during

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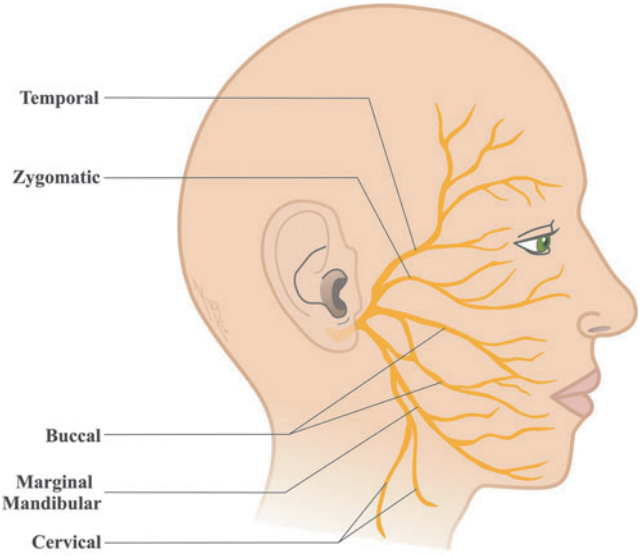

FIG. 1. Anatomy of the human facial nerve and the five main branches as temporal, zygomatic, buccal, marginal mandibular, and cervical.

temporomandibular joint replacement, mastoidectomy, parotidectomy, and rarely during several cosmetic procedures, including face lift.[7–9] Iatrogenic injuries range from simple transection of the main trunk or peripheral branches to varying degrees of segmental loss.[10]

Management options

Considering the anatomical and etiological differences, management of the facial nerve injury can be more complex than other peripheral nerves.[11,12] The decision for surgical intervention is usually reserved for patients with an anatomical disruption of the nerve, or who are unlikely to make a satisfactory recovery following medical treatment, or chronic complete paralysis.[1,2,6,9,10,13] Tension-free primary repair of the divided nerve yields the best results, when possible (Fig. 2). If primary repair is not possible, autografts remain the standard of care for facial nerve defects with gaps. When the proximal and distal ends of the facial nerve are available, graft interposition, commonly using the sensory nerves as donor, such as great auricular or sural nerve, can be performed.[14–16] When the proximal nerve end is absent, options for dynamic procedures become much more challenging with poorer outcomes. Reconstruction might require the use of alternative muscles for reanimation or nerve transfers, including hypoglossal, accessory, and masseteric nerves, as well as cross-face nerve grafts.[17–23] In cross-face nerve grafting, a facial nerve branch from the healthy side of the face is used to reanimate the paralyzed side of the face by connecting the two with a nerve graft, such as autologous sural nerve. Cross-face nerve grafting using an autologous sural nerve is commonly performed in combination with hypoglossal or masseteric nerve transfer.[24–27] However, these procedures result with donor site complications such as scarring and loss of sensation.

Bioengineering approaches to facial nerve repair

Bioengineering plays an important role to create artificial materials that are able to mimic the nerve itself without the need for a donor nerve.[28,29] With the concordance to the key design parameters of a useful nerve guide or conduit,[30] there are various applications of biomaterials and decellularized allografts to find the best option to enhance recovery compared to the gold standard autografts.[31–33] These biomaterials range from simple silicone conduits to more complex and novel technologies, including nanocomposite-coated silk-based[34] conduits. In addition, various cues, such as neurotrophic factors,[35] stem cells,[36] or adipose cells,[37] inside the conduits to induce neural recovery are being increasingly studied in preclinical models. While there are limited clinical studies examining facial nerve repair using nerve conduits, early results are promising. For example, a poly(glycolic acid) (PGA) tube was used to repair posttraumatic lesions of the facial nerve in seven patients with gap sizes ranging between 1 and 3 cm.[38] Compared to the contralateral side, muscle recovery was >60% in one patient, up to 60% in four patients, and at 30% in two patients. Two additional case reports described PGA/collagen conduits as a promising alternative treatment for facial nerve

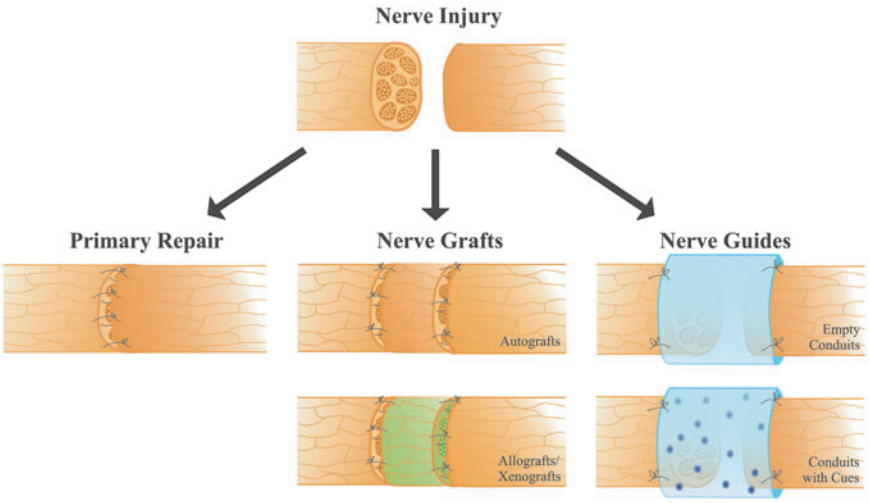

FIG. 2. Different facial nerve repair methods, including primary repair, and the use of nerve grafts or conduits.

recovery.[39,40] In two patients with a nerve gap of 16 and 20 mm, frontal muscle movement showed recovery at 5 months following repair.[39]

Allografts, from a different organism within the same species, and xenografts, from a different species, can also be considered options. Processed nerve allografts are another promising alternative treatment for facial nerve repair.[41–43] Although their use is more common and established in peripheral nerve repairs, studies are limited on the facial nerve.[44] Safa *et al.* reported two cases of improved meaningful sensory recovery and four cases of improved meaningful motor function using a processed nerve allograft in the head and neck region.[42] While no comparison is currently available due to the limited sample size in this region, the initial results are promising. Allografts are also more commonly used in the repair of the trigeminal nerve (e.g., inferior alveolar nerve) to achieve sensory recovery.[45–49]

This review focuses on preclinical models that study facial nerve repair with bioengineered materials, including conduits and nerve grafts (allograft, xenograft, and autograft).

Overview of Outcome Measurements in Preclinical Models

Preclinical studies remain as the hallmark for the development of novel bioengineering approaches and translation of these developments into the clinical practice. The majority of the studies use rodent models while studying the facial nerve recovery. Table 1 summarizes outcome measures used in these studies. The main focus of this section is the functional outcome measures. Establishing an appropriate model with accurate outcome measures for the aims that are being studied is crucial to quantify recovery of the facial nerve and analyze functional outcomes. Movements of the facial muscles (whisking, eye closure, and ear movements) that are innervated by their corresponding nerve branches are commonly used as functional measures[50] and described in detail in the following section. Histological analysis, including nerve sections, retrograde tracing of neuron bodies, and motor endplate reinnervation measures are also briefly described.

Whisking

Due to similar facial nerve innervation between humans, rodents, and rabbits, tracking the whisking movements provides a clinically relevant model regarding the degree of compromise of the facial nerve, following an intervention.[51–53] The motor activity of protraction and retraction movements of the vibrissae in rodents is controlled by the pilo-erector muscles and innervated mainly by the buccal branch of the facial nerve (Fig. 3).[54–56] Whisker movement, symmetry, amplitude, frequency, and other whisking behaviors are indicators of facial nerve functional recovery through innervation of superficial muscles controlling whisking.[57] However, the overlap of different efferent motor and autonomic supply to the whiskers should also be considered, while assessing facial nerve regeneration.[58–60] In addition, transection and ligation of the ends of the marginal mandibular branch is also used in models focusing on whisking to prevent dual innervation of the buccal and mandibular branches on the pilo-erector muscles.[61–63]

Whisking measurements in the studies are also used to monitor the progression of recovery over specified time scales. Although the time for the measurement of the functional outcome changes depending on the design, an ideal time to evaluate facial nerve recovery by whisking in rats was found to be within the 4 months after the injury.[64] Current whisking protocols range from gross observation, to surgical implantation of restrictors coupled with laser micrometer setups. One method to objectively analyze whisking movement was first described by Heaton *et al.*, for simultaneously monitoring bilateral eyelid and whisker movements in a rat model, where rats were secured in a full body restraint device and surgically fitted with titanium head implants to provide rigid head fixation points.[57] However, using surgical implants for rat head fixation raises the possibility for infection, and may affect normal whisking behavior due to the rigid restraining practice. Therefore, to avoid this, other authors have developed parameters to monitor rat health and behavior before continuing with experimentation. Chen *et al.* allowed 2 weeks for recovery following implantation of the head fixation device, and gradually introduced rats to the restraining apparatus to reduce its impact on the normal whisking behavior of the

Table 1. Overview of Different Outcome Measures in Rat Studies

	Outcome measures	*References*
Functional	Scoring systems	62,63,66,67,70–72,78,81,87,106,117
	Whisker movements	58,61,65,83,85,88,90,97,105,110,115,157–159
Electrophysiological		58,61,63,66,71–75,77,78,89,90,95–98,103,104,109,110,157–160
Histological	Toluidine blue staining	61,65,66,74,76–79,86,87,89,91,95,96,98,103,106,108–110,115,116,158,159
	Transmission electron microscopy	61,62,73–75,77,78,86,87,89,91,96,98,103,104,109,116,157
	Scanning electron microscopy	75,115
Immunohistochemical	S-100 antibody stain	75,76,78,87,89,103,111,158
	Neurofilament antibody stain	58,72,75,78,111
	Tubulin antibody stain	81,117,158
	Other antibody stains (i.e., Tuj-1, GAFP, NGF, acetylcholine transporter, Synaptophysin, VIP)	58,66,76,87,89,91,104
Retrograde neuronal tracing	Fluorescence labeling	61,65,71,73,74,76,79,81,88,96,98,108,110,115–117
	Horseradish peroxidase labeling	104

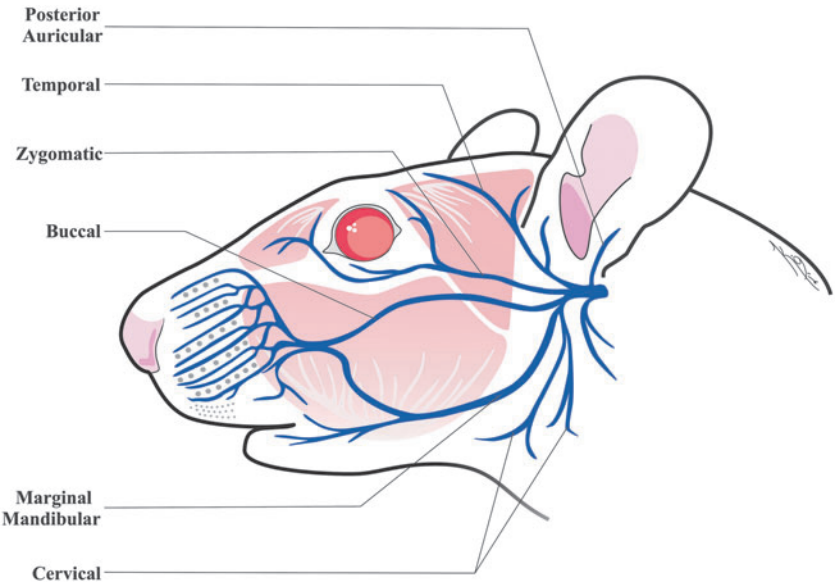

FIG. 3. Anatomy of the rat facial nerve.

rats.[65] Abbas *et al.* developed a custom-made restraining device that only secured the body and left the head free to move around.[66] To ensure quality and consistency of filming, despite the free range of motion in the rat heads, rats were recorded for a longer duration and the most optimal portions of the recording were chosen for the analysis.

Scoring systems and eye closure

Scoring systems in facial nerve studies often measure similar parameters to those in whisking studies, such as facial symmetry, whisking movement, and whisker symmetry, and create a numeric scale for researchers to score these behaviors.[67] In addition, scoring systems normally do not employ a restraining apparatus and instead allow animals to roam free in a cage or transparent box, while being filmed or observed.

Eye closure, which is controlled by the orbicularis oculi muscle and mainly innervated by the zygomatic branch of the facial nerve in rodents, is commonly used together with whisking in qualitative scoring systems.[50,57,68,69] Monitoring eye and whisker movement simultaneously results in a more comprehensive outcome measure as the facial nerve innervates a wide range of facial muscles in rodents and other animal models.[51] Yasui *et al.* measured recovery by using a scoring system in a rat model with symmetry of the eyes at rest, eye closure capability, symmetry of the vibrissae at rest, and motion of the vibrissae compared to the untreated side.[70] Li *et al.* utilized gross observation and a five-point scoring system in a rat model involving eye closure, blinking reflex, and whisking.[71]

Electrophysiology

Electrophysiological measurements are widely utilized to assess facial nerve function in preclinical models to provide insight into the regenerative progress of the impacted nerve. This can be considered a distinct outcome measure compared to other functional measures. One of the major limitations is that it does not address changes that might have occurred with muscle physiology and function due to the loss of innervation during the regenerative period. Therefore, many groups have used a combination of electrophysiological and whisking/scoring systems.[66,70,72]

Electrophysiology is ideal for identifying specific nerve defects when the impacted nerve is not necessarily known. As the facial nerve has a wide variety of innervated muscles, gross observations of facial dissymmetry and function may not be able to identify the specific branch of the impacted nerve.[51–53] Electrical function of specific branches of the facial nerve can be determined using electrophysiology and measuring muscle action potentials.[73] Another practical use is the ability to compare latency, amplitude, and duration of compound muscle action potentials between different methods of facial nerve regeneration procedures.[74,75]

Histological analysis

The majority of the studies use regenerating axon count, myelinated axon count, fiber diameter, axon diameter, myelin thickness, or their variations, to quantify the regenerative capacity of the experimental groups and analyze histological outcomes. A commonly used method is osmium tetroxide-toluidine blue staining, where slides can be viewed both under light microscopy or transmission electron microscopy.[76–78] In addition, immunohistochemical staining is used mainly with antibodies against S-100 for Schwann cells, neurofilaments for axon cytoplasm or tubulin.

Retrograde labeling of motor neurons is another method performed to understand the degree of axonal branching both qualitatively and quantitively.[50] This allows visualization of target muscle and estimation of motor end-plate reinnervation. Fluorescence staining methods are performed by injecting tracers such as Fluoro-Gold,[76,79,80] 1,12-dioctadecyl-3,3,3′,3′-tetramethyl indocarbocyanine perchlorate (DiI),[77] Fast Blue,[81] cholera toxin subunit B conjugate,[73] or triple staining[82] into the whisker muscles, a few days before the sacrifice. After allowing the agents to travel in a retrograde manner, the labeled motor neurons in the facial

nerve nucleus in brainstem sections can be counted under fluorescent microscope. In addition, macroscopic *in vivo* imaging in *Thy1-GFP* rats expressing green fluorescent protein in their neural structures can be performed to trace the distal end of the regeneration.[79,83]

Rat Models of Facial Nerve Defects

Majority of preclinical studies use rat models to study facial nerve repair. Three primary types of nerve injury models are used as nerve crush, transection, and nerve gap.[84,85] Facial nerve defect/gap sizes vary between the models, but generally remain <10 mm gap for conduit studies. The vast majority of conduit studies are in the range of 5–8 mm gaps with several nerve transection-only studies, and there are limited applications in larger gaps. Graft studies (allografts and autografts), on the other hand, use models with gaps larger than 10 mm, such as cross-face neurotization.

Permanent conduits

Several studies have used silicone tubes as a vehicle to locally apply and entrap different factors that stimulate nerve regeneration (Table 2). For example, Matsumine *et al.* delivered fibroblast growth factor (FGF-2) into a 7 mm defect in the facial nerve; the delivery system included FGF-2 embedded in microspheres inside a silicone conduit.[86] The FGF-loaded microsphere group facilitated recovery with increased nerve regeneration. Therapeutic potential of adipose stem cells was identified in a model by embedding the cells in a collagen gel that was transplanted into the 7 mm gap in the rat facial nerve, inside a silicone tube.[87] Mature adipocyte-derived pluripotent cells demonstrated potential in promotion of maturation of the regenerated nerve in a similar rat model.[77] Stromal vascular fraction, a source of adipose stem cells, was infused into silicone tubes and promoted nerve regeneration with better histological and functional outcomes compared to conduit alone.[62] Dental pulp cells, as a derivative of neural crest and a source of Schwann and neural progenitor cells, were used to accelerate rat facial nerve recovery, and demonstrated positive effects both histologically[76] and functionally[63] with silicone conduits in a 7 mm gap. Finally, olfactory ensheathing cells (OEC), as a source of glial cells and trophic factors, were used inside silicone tubes to stimulate axonal growth and sprouting in rat facial nerve injury.[88]

Biodegradable conduits

Biodegradable nerve conduits include type I collagen, PGA, poly(DL-lactide-co-caprolactone), poly(DL-lactide-co-glycolide) (PLGA), poly(lactic acid) (PLA), poly (caprolactone) (PCL), and nonresorbable poly(vinyl alcohol). PLA conduit demonstrated enhanced nerve regeneration than silicone tubes, and comparable results with the gold standard of autologous nerve grafting in a 7 mm gap model.[89] PLA/chitosan conduit was also used in combination with OEC to repair a 5 mm defect in the facial nerve, and olfactory mucosal cells were found to be more effective compared with the olfactory bulb.[71] PLGA nerve conduit was used in a 5 mm facial nerve defect model, which contained Schwann cells transfected with glial cell line-derived neurotrophic factor (GDNF).[90] The GDNF-treated group performed better than nontransfected cells and primary anastomosis. PLGA nerve conduit was used in another study of 7 mm nerve defect in the buccal branch of the facial nerve in combination with dental pulp cells and promoted nerve regeneration.[91] PCL/collagen conduit was used in a 4 mm gap model, combined with human umbilical cord serum, and yielded more favorable results compared with the autograft.[78]

PGA is among the first clinically available and most successful material implanted in humans for peripheral nerves.[29,92–94] PGA has been widely studied in rat facial nerve models.[28] For example, Costa *et al.* demonstrated that PGA tubes filled with bone marrow-derived Schwann-like cells were associated with superior nerve regeneration in a 5 mm gap.[95] Effects of both adipose stem cells and stromal vascular fraction have also been demonstrated in nerve regeneration with PGA tubes in a 7 mm gap.[96] Fujimaki *et al.* identified that PGA tubes filled with cells cultured from mature adipocytes enhanced regeneration and improved physiological function in a 7 mm gap.[61] Human olfactory stem cells were used inside PGA tubes for a 2 mm gap with accelerated nerve regeneration.[97] Also, collagen-coated PGA conduit was found to be more effective compared to nerve autograft in hypoglossal-facial nerve connections in an incomplete rat facial nerve paralysis model.[98]

Three-dimensional printed constructs

A novel approach in tissue engineering and regenerative medicine is three-dimensional (3D) bioprinting of nerve guidance constructs.[99] This allows customization of the shape or material of the nerve guides or scaffolds to be used to repair complex defects with bifurcating motor or sensory branches.[100] Various cues, including growth factors or stem cells, can be combined with this process to improve physical and chemical properties of the constructs and enhance the customized neuroregenerative functionality.[101] In addition, scaffold-free constructs can also be created solely based on stem cell components. For example, Zhang *et al.* used a scaffold-free bioprinting methodology to create constructs from gingiva-derived mesenchymal stem cell spheroids and transplanted to a 5 mm defect in the buccal branch of the rat facial nerve.[102] The functional recovery was better than the simple silicone tubes, yet not as great as the autografts. However, the histological organization of the nerve fascicles and the target muscle recovery achieved through electrophysiology was similar to the autografts, highlighting the promising future applications to promote nerve regeneration.

Nerve grafts

Autografts and allografts have a broad range of applications in both preclinical and clinical studies (Table 3). Sources of grafts include peroneal, median, and sciatic nerves. In a 10 mm gap model of the buccal branch of the facial nerve, three graft types (autogenic peroneal nerve graft, acellular facial nerve graft from another rat, and acellular facial nerve graft from a rabbit) were compared.[103] Autografts demonstrated the strongest regenerative capacity, whereas allografts and xenografts had similar effects. In another study, acellularized xenografts from rabbits were

Table 2. Overview of Preclinical Models Using Conduits

References	Model	Gap length (mm)	Branch	Conduit type	Cues being studied inside the conduit
159	Rat	6	Buccal branch	NA	PLOD1 depleted adipose-derived stem cells
61	Rat	7	Buccal branch	PGA/collagen	Dedifferentiated fat cells
98	Rat	Crush injury	Main trunk	PGA/collagen	Interpositional-jump graft
71	Rat	5	Main trunk	PLA/chitosan	Olfactory ensheathing cells
96	Rat	7	Buccal branch	PGA/collagen	Adipose-derived stem cells or stromal vascular fraction
75	Rat	7.5	Buccal branch	Peptide amphiphile nanofiber/collagen	NA
157	Rat	8	Buccal branch	Collagen	Heparin, basic fibroblast growth factor, neural stem/progenitor cells
97	Rat	2	Main trunk	PGA	Neural stem cells originating from the human olfactory mucosa
78	Rat	4	Main trunk	PCL/collagen	Human umbilical cord serum
62	Rat	7	Buccal branch	Silicone	Syngeneic uncultured-stromal-vascular-fraction
74	Rat	Crush injury	Main trunk	Silicone/collagen	Interpositional-jump graft
77	Rat	7	Buccal branch	Silicone/collagen	Dedifferentiated fat cells
63	Rat	7	Buccal branch	Silicone/collagen	Collagen gel embedded with dental pulp cells
86	Rat	7	Buccal branch	Silicone	Acidic gelatin hydrogel microspheres with basic fibroblast growth factor
87	Rat	7	Buccal branch	Silicone/collagen	Undifferentiated adipose-derived stem cells, differentiated adipose stem cells, Schwann cells
95	Rat	5	Mandibular branch	PGA	Bone marrow stroma mesenchymal stem cell-derived Schwann-like cells
89	Rat	7	Buccal branch	PLA	Type 1 collagen solution
85	Rat	2	Main trunk	Silicone	NA
90	Rat	5	Buccal branch	PLGA	Schwann cells transfected with GDNF
88	Rat	5	Main trunk	Silicone	Olfactory ensheathing cells
76	Rat	7	Buccal branch	Silicone	Collagen gel embedded with dental pulp cells
91	Rat	7	Buccal branch	PLGA	Collagen gel embedded with dental pulp cells
124	Rabbit	Crush injury	Buccal branch	Collagen	Collagen-binding domain basic fibroblast growth factor
121	Rabbit	10	Buccal branch	Chitosan	Nerve growth factor microspheres
120	Rabbit	10	Buccal branch	Chitosan/collagen	Nerve growth factor, neural stem cells
123	Rabbit	6	Buccal branch	PGA	FK506 dissolved in olive oil
122	Rabbit	10	Buccal branch	Polytetrafluoroethylene/ collagen	NA
119	Rabbit	8	Buccal branch	Silicone	Nerve growth factor containing solution
118	Rabbit	8	Buccal branch	Silicone	Nerve growth factor containing solution
142	Feline	0	Main trunk	Collagen	Sterile normal saline
141	Feline	5	Dorsal ramus	Collagen	None
149	Minipig	35	Buccal branch	Collagen/nano-sized b-tricalcium phosphate	Nerve growth factor
148	Minipig	35	Buccal branch	Collagen	Linear ordered collagen scaffold combined with recombinant proteins
147	Minipig	10	Buccal branch	Collagen	Collagen-binding ciliary neurotrophic factor absorbed linear ordered collagen fibers

GDNF, glial cell line-derived neurotrophic factor; NA, not available; PGA, poly(glycolic acid); PLA, poly(lactic acid); PCL, poly(caprolactone); PLGA, poly(DL-lactide-co-glycolide).

used to repair a 6 mm gap in the facial nerve of rats and yielded comparable regenerative capacity with autografts.[104]

Many of the previously mentioned rat studies used either reverse-polarity autografts or a donor nerve from a distant site as their control groups to compare with conduits. For example, Hohman *et al.* created a 20 mm gap to compare reverse-polarity autografting with transection and primary repair of the facial nerve.[105] Although initially slower, recovery after autografting was similar to the primary repair. In terms of the type of the autograft as sensory or motor branch, histological and physiological outcomes were similar in a 5 mm gap model in the main trunk of the facial nerve.[72]

Different surgical methods have been tested with autografts.[106] End-to-side loop grafting is a technique used to reconstruct multiple branches by allowing the distal stump of the facial nerve to be connected to the single transplanted nerve.[107] Matsumine *et al.* incorporated the concept of double innervation into the end-to-side loop grafting by using a contralateral buccal branch of the facial nerve.[108] Li *et al.* used a predegenerated peroneal nerve graft to connect ipsilateral hypoglossal or accessory nerves with the facial nerve.[73] Matsumine *et al.* showed that vascularized median nerve grafts, where the median nerve was transferred to the defect site with the median artery and vein, in a 7 mm gap model yielded better histological and functional nerve regeneration when compared with nonvascularized graft.[109] Adipose-derived stem cells were tested in another study by injecting into the local environment of the cross-face nerve graft and resulted in enhanced recovery.[66]

End-to-side cross-face nerve grafting has been applied in a 15 mm gap model with improved functional recovery.[110] In another study, Placheta *et al.* used peroneal nerve as a cross-face nerve graft enhanced with end-to-side coaptation of sensory occipital nerves.[79] This resulted in improved regeneration and functional outcomes due to the protective effects on chronic denervation. These models are infrequent in rats as they study repairs in longer gaps compared with models that study conduits.

Blood vessels

Autogenous vein and artery grafts are used to provide an enhanced environment for the axonal regeneration, while acting as sturdy barriers against scar ingrowth.[111–114] However, in a model of transection of the facial nerve trunk, autologous venous ensheathment failed to improve the quality of regenerated axons in terms of histology and function.[65] In another study, decellularized allogenic artery graft was used in a 6 mm gap as a conduit filled with adipose stem cells, where addition of adipose stem cells facilitated the recovery, however, to an extent lower than nerve autografts.[115,116] The abdominal aorta and its bifurcations were used as a Y-shaped conduit in a large facial nerve defect, where although the Y-tube improved axonal pathfinding, the functional outcome did not improve.[81,117]

Rabbit Models of Facial Nerve Defects

Rabbit models are commonly used for larger defects (>10 mm), given that the larger scale of the animal and ample amount of facial nerve length compared with rodents. However, unlike rat models, there are limited numbers of rabbit models of facial nerve repair. The following sections describe some of these studies.

Permanent and biodegradable conduits

Several studies have examined silicone tubes in rabbit facial nerve repair. For example, in an 8 mm nerve gap, silicone tubes were used as conduits that contain nerve growth factor (NGF) solutions.[118,119] Conduits with growth factor were associated with fewer collateral nerve sprouts, and the functional outcome was comparable with autologous nerve grafts. Guo *et al.* combined neural stem cells and NGF in a chitosan conduit and protein sponge to recover a 10 mm defect in the facial nerve, where neural stem cells yield better recovery.[120] Liu *et al.* used NGF releasing microspheres for the local administration in a chitosan conduit.[121] Sustained release of NGF from the microspheres significantly improved the outcomes when compared with the injection of NGF into the conduits.

Expanded polytetrafluoroethylene and collagen tubes were used in a 10 mm gap model with similar outcomes with the autologous nerve grafts in the long term.[122] Diaz *et al.* applied FK506 locally at the time of repair within a PGA tubing into a 6 mm defect, where superior results in nerve regeneration were achieved versus interposition autografts.[123] In another study, collagen nerve conduits with recombinant proteins were used in a cut or crush model with promising outcomes.[124]

Nerve grafts

Nerve grafts have various uses in models with longer gaps and different surgical techniques. In a whole facial nerve defect model of 20 mm gap, Hu *et al.* identified that whole facial nerve allograft was efficient in recovery when the defect was repaired with acellular facial nerve allografts, autologous facial nerve grafts, acellular peroneal nerve allograft, or autologous peroneal nerve grafts.[125] Although the difference between acellular and autologous grafts was minimal, facial nerve grafts performed better than peroneal nerve grafts. Another study compared vascularized versus nonvascularized median nerve grafts in a 10 mm nerve gap in the intratemporal part of the facial nerve.[126] Vascularized grafts were associated with superior outcomes in a bony recipient bed. Neural cable graft, where the resected segment of 10 mm was rotated 180° (i.e., reverse-polarity autograft), was used with the prefabrication process of antecedent crush injury before grafting.[127] Antecedent injury was associated with enhanced innervation of the mimetic muscles. In another model, a 5 mm segment was resected and repaired using sural nerve graft by either classical suturing or a tissue adhesive, where the tissue adhesive was found to be ineffective.[128]

The auricular nerve of the rabbit that courses along the ear serves as a donor site for autograft harvesting. Predegenerated auricular nerve grafts by crush injury were used in a complete buccal branch defect with comparable outcomes.[129] Auricular nerve graft was also used as vascularized or nonvascularized nerve grafts in a 20 mm segmental gap model.[130] Vascularized nerve graft was associated with greater numbers of regenerated axons and Schwann cells due to rapid reestablishment of blood circulation in the graft.

Table 3. Overview of Preclinical Models Using Grafts

References	Model	Gap length (mm)	Branch	Graft type	Graft details	Additional treatments	Outcomes
[72]	Rat	5	Main trunk	Autograft	Motor branch to the quadriceps muscle, sensory femoral cutaneous branch	NA	Similar results between sensory and muscle autografts
[65]	Rat	Transection	Main trunk	Venous autograft	Retromandibular vein	NA	Autologous venous ensheathment did not improve recovery
[66]	Rat	Cross-face nerve grafting	Main trunk and buccal branch	Autograft	Sciatic nerve	Adipose-derived stem cells	Adipose-derived stem cells enhanced recovery
[103]	Rat	10	Buccal branch	Allograft, xenograft, autograft	Acellularized facial nerve, acellularized facial nerve from rabbit, peroneal nerve	NA	Autografts performed better than acellular nerve grafts, allografts and xenografts had similar effects
[79]	Rat	Cross-face nerve grafting	Marginal mandibular and buccal branches	Autograft	Peroneal nerve	End-to-side coaptation of sensory occipital nerves to the graft	Sensory pathway protection improved recovery
[104]	Rat	6	Buccal branch	Xenograft, autograft	Acellularized facial nerve from rabbit, facial nerve	NA	Similar results between xenografts and autografts
[109]	Rat	7	Buccal branch	Autograft	Vascularized and nonvascularized median nerve	NA	Vascularized median nerve graft resulted in improved recovery
[105]	Rat	20	Marginal mandibular and buccal branches	Autograft	Reversed facial nerve	NA	Recovery after cable grafting is slower, but eventually similar compared to primary neurorrhaphy
[108]	Rat	>10	All major branches	Autograft	Contralateral facial nerve buccal branch	Hypoglossal nerve supercharging	Multiple branches were recovered, axonal supercharge from the hypoglossal nerve improved recovery
[160]	Rat	Transection	Not specified	Amniotic membrane	From a donor bank	NA	Amniotic membrane covering improved recovery
[81]	Rat	>10	Marginal mandibular and cervical branches	Arterial allograft	Abdominal aorta and its bifurcation	NA	Y-tube reconstruction decreased axonal branching, but did not improve recovery
[116]	Rat	8	Buccal branch	Arterial allograft	Acellularized abdominal aorta	Autologous transdifferentiated adipose-derived stem cells	The use of adipose-derived stem cells improved recovery, but was inferior to the autografts.

(*continued*)

TABLE 3. (CONTINUED)

References	*Model*	*Gap length (mm)*	*Branch*	*Graft type*	*Graft details*	*Additional treatments*	*Outcomes*
[117]	Rat	>10	Marginal mandibular branch	Arterial allograft	Abdominal aorta and its bifurcation	NA	Y-tube reconstruction decreased axonal branching, but did not improve recovery
[110]	Rat	Cross-face nerve grafting	Bilateral all major branches and main trunk	Allograft	Sciatic and ulnar nerve	NA	End-to-side nerve grafting improved recovery
[130]	Rabbit	20	Buccal branch	Autograft	Vascularized and nonvascularized central auricular nerve	NA	Vascularized auricular nerve graft resulted in improved recovery
[129]	Rabbit	>10	Buccal branch	Autograft	Predegenerated and normal great auricular nerve	NA	Predegenerated grafts resulted in improved recovery
[132]	Rabbit	10	Buccal branch	Venous autograft	Ipsilateral facial vein	Transdifferentiated Schwann-like MSC and bone marrow MSC	Transdifferentiated Schwann-like MSC improved recovery
[125]	Rabbit	20	All major branches and main trunk	Allograft, autograft	Acellularized facial nerve, facial nerve, acellularized peroneal nerve, peroneal nerve	NA	Both facial nerve autografts and allografts performed similar, and better than peroneal nerve grafts
[131]	Rabbit	10	Buccal branch	Venous autograft	Standard and inside-out ipsilateral facial vein	NA	Inside-out vein graft accelerated recovery
[53]	Rabbit	10	Intratemporal segment	Autograft	Vascularized and non-vascularized median nerve	NA	Vascularized median nerve graft resulted in improved recovery in a bony recipient bed
[153]	Monkey	30–40	Middle zygomatic branch	Autograft, allograft	Superficial fibular nerve	Systemic FK506	Autografts performed better in terms of neuron number and electrophysiological recording, but both allografts and autografts achieved similar functional results
[146]	Canine	20	Palpebral branch	Autograft	Superficial peroneal nerve	NA	End-to-side coaptation accelerated recovery
[145]	Canine	Cross-face nerve grafting	Multiple branches	Autograft	Sural nerve	Pulse generator to continually stimulate the muscle	Improved recovery in stimulated muscles

MSC, mesenchymal stem cell; NA, not available.

Blood vessels

There are a handful of studies that describe the use of autogenous vein grafts in rabbit facial nerve repair. For example, Tang *et al.* compared autologous vein grafts and vein grafts that are turned inside-out in a 10 mm segmental nerve gap.[131] Although both vein grafts were beneficial for nerve regeneration, the inside-out vein grafts were associated with accelerated axonal regeneration. Vein grafts were also used as conduits to deliver stem cells in a 10 mm segmental nerve defect.[132]

Other Animal Models of Facial Nerve Defects

Murine

There are different surgical models to study the facial nerve injury and repair in mice.[133,134] Several models were also used to understand the molecular mechanisms of the facial nerve regeneration after injury.[135–140]

Feline

Cat models have been used to assess novel nerve guides as the representation of facial muscles in the facial nucleus resemble those in humans and to assess eyelid responses. For example, Kitahara *et al.* demonstrated that collagen nerve guide in a 5 mm gap in the facial nerve was associated with enhanced recovery.[141] Dresner *et al.* also demonstrated that a collagen conduit resulted enhanced regeneration compared to without conduits, after transection of the main trunk of the facial nerve.[142] In another study with a 5 mm defect in the cat facial nerve, alginate was used as a promising material for the repair without suturing.[143] The adaptability and plasticity of the facial motor system was also assessed in a study with different surgical approaches.[144]

Canine

In a study examining effects of continuous electrical stimulation of the denervated muscles, a beneficial effect was demonstrated compared with nonstimulated controls in a canine model of complete facial nerve paralysis that was repaired with cross-facial nerve grafts using two donor sural nerves (20–25 cm in length).[145] End-to-side coaptation was found to be effective in producing synchronous blink in a canine facial nerve defect model that was reinnervated with the contralateral side by a peroneal nerve graft.[146]

Minipig

Minipig models of larger gaps were used with collagen nerve conduits to deliver certain cues to the defects. In an 8 mm nerve gap model, collagen-binding ciliary neurotrophic factor-absorbed fibers were found to have promising results.[147] Cui *et al.* also studied a collagen scaffold combined with recombinant proteins in a 35 mm gap model with favorable outcomes.[148] In a similar 35 mm gap model, type 1 collagen and nano-sized beta-tricalcium phosphate conduits were examined and combined with NGF.[149] Groups with NGF were found to be associated with promoted nerve regeneration when compared to the groups without NGF.

Ovine

Ovine models were identified as readily available and affordable models for training in head and neck surgery, with similarities in skin, subcutaneous, and bony structures seen in human dissections.[150] Glasby *et al.* studied the repair of 5 cm gaps with freeze-thawed, coaxially aligned skeletal muscle autografts, where muscle grafts were found to be favorable when compared with other surgical techniques.[151] Another model studied ciliary neurotrophic factor injection to the muscle and found that it was not effective in the repair of buccal branch of the facial nerve.[152]

Monkey

In a monkey model, 3–4 cm of the middle zygomatic branch of the facial nerve was removed and repaired with autografts and cold preserved nerve allografts temporarily treated with FK506, and the animals received oral FK506.[153] Autografts performed better in terms of neuronal counts and electrophysiological recordings, but both allografts and autografts achieved similar functional results.

Conclusions and Future Prospects

Recovery after facial nerve injury is a complex and multifactorial process. Tissue engineering plays an important role in the recovery to achieve the best possible outcome in challenging clinical scenarios without any extra morbidity in the patients. Results of studies using biomaterials for nerve repair compete, or even enhance recovery compared to the gold standard autografts. The use of cues inside the conduits such as neurotrophic factors or stem cells is an ever-expanding area with exceptional promise to achieve enhanced recovery. However, a consensus on the ideal cell source, neurotrophic factor, and biomaterial is yet to be reached.

Current preclinical models on facial nerve repair are well established with the major focus on rodents. The majority of the studies focusing on bioengineered conduits use relatively smaller nerve gaps, whereas larger gaps in larger animal models are mainly the focus of nerve graft studies (such as cross-face nerve grafting). The effects of conduits on the challenging large gaps need to be further explored. While there are currently limited clinical studies examining facial nerve repair using nerve conduits or processed acellular nerve allografts, early results are promising. Combinatory approaches of stem cells and different treatment modalities with the processed nerve allografts are yet to be applied for the facial nerve repair.[154] In addition, further research in the field of 3D bioprinting will allow translation of an individually tailored approach for complex facial nerve injuries. An ideal recovery method could potentially be of major benefit in a wide range of areas concerning with the facial nerve repair, from facial transplantations[155,156] to trauma or cancer surgery.

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