JPRAS Open 44 (2025) 145–161

Contents lists available at ScienceDirect

JPRAS Open

journal homepage: www.elsevier.com/locate/jpra

Adipose-derived stem cells in fat grafting for facial paralysis: A review of their therapeutic modality ☆

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ARTICLE INFO

Article history:
Received 19 October 2024
Accepted 25 January 2025
Available online 17 February 2025

Keywords:
Facial paralysis
Facial palsy
Stem cells
Adipose-derived stem cells
Fat grafting

ABSTRACT

Facial paralysis, characterized by the complete loss of voluntary muscle control in the face, significantly affects individuals' daily lives. Recently, stem cell therapy has gained attention as a potential treatment for various medical conditions due to its capacity for self-renewal and differentiation into specialized cell types. Adipose-derived stem cells (ADSCs), obtained from the stromal-vascular fraction, consist of mesenchymal stem cells (MSCs), smooth muscle cells, endothelial cells, pericytes, lymphocytes, and tissue macrophages. Both differentiated and undifferentiated MSCs support axonal regeneration, enhance motor function,

☆ **Declarations of work presentation** This work has been submitted as an abstract and presented as an e-poster at the 33rd Congress European Academy of Dermatology and Venereology in Amsterdam, 25–28 September 2024 under the following title "The Therapeutic Potential of Adipose-Derived Stem Cells in Fat Grafting for Facial Paralysis: A Review."

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https://doi.org/10.1016/j.jpra.2025.01.019

and promote growth factor release. ADSCs have been shown to improve regenerative outcomes, including better axonal development, increased myelinated fiber count, greater myelin thickness, and enhanced target reinnervation. These cells can differentiate into various lineages, particularly Schwann-like cells that facilitate axon regeneration. Additionally, ADSCs play a role in healing peripheral nerves by releasing neurotrophic and angiogenic factors. While the results are not as effective as nerve autografts, ADSCs offer an alternative option for reconstructing facial nerve paralysis.

Introduction

Facial palsy (FP), or facial paralysis, is a condition that can happen due to either the partial or complete loss of voluntary muscle function in the face. The individuals who suffer from this condition are faced with serious issues that affect their lives and psychological well-being. Facial paralysis epidemiology reveals the multifactorial nature of this disease, describing different origins ranging from birth defects to trauma, infection, and neurologic disorders.[1]

The treatment of facial paralysis predominantly involves surgical operations, physical therapy, and supportive measures such as facial slings or botulinum toxin injections. While these treatments can lead to certain functional improvements and provide support in some cases, most are largely symptomatic and do not restore natural movement and expression. In contrast, reconstructive surgical procedures, including nerve grafts or muscle transfers, aim to achieve more significant results, though they can sometimes result in complications. These challenges underscore the need for novel therapeutic strategies.[2]

Managing long-term functional disability in patients with chronic facial nerve palsy poses a significant challenge for clinicians. The intricate nature of reconstructive surgery aimed at restoring facial motor function is apparent. Despite significant advancements in nerve grafting and multidisciplinary surgical care, only around 60% of these patients attain a favorable cosmetic outcome.[3]

In recent years, stem cell-based applications have emerged as promising approaches for addressing various medical conditions due to the unique abilities of stem cells to restore, regenerate, and deliver immunomodulatory functions. Adipose-derived stem cells (ADSCs) have garnered significant research interest because of their abundance, ease of isolation, and high replication potential. These multipotent cells function like a well-organized army, capable of differentiating into adipocytes, osteocytes, chondrocytes, and myocytes, making them particularly suitable for tissue regeneration and repair.[4] The role of stem cells extends beyond the treatment of facial paralysis, encompassing a wide range of medical fields where their effectiveness is well recognized. Specifically, in the context of facial paralysis, ASC-based treatments offer a novel approach for regenerating nerve fibers, reinnervating muscles, and remodeling facial structures.[4] In this paper, we explored the use of ADSCs through fat grafting as a promising therapeutic modality for the treatment of facial paralysis.

Methodology

For the study, we collected information from several research studies. We used these keywords during our search for data: “facial paralysis” and “adipose-derived stem cells” and “facial palsy” AND “fat grafting” through PubMed, Scopus, and ScienceDirect databases. We included articles related to ADSCs and fat grafting/injection/transfer in cases with facial paralysis and excluded articles that involved surgical nerve repair. After going through 622 articles and applying inclusion and exclusion criteria, 15 articles were selected. After a careful review of all their references and following the

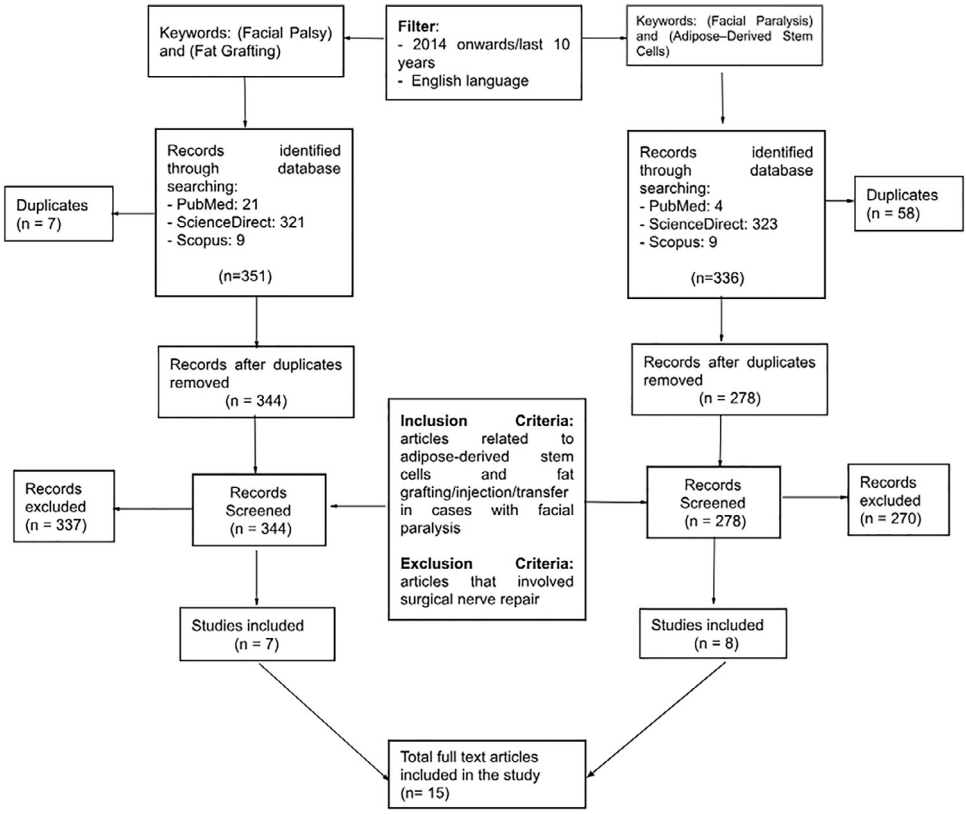

Figure 1. Article selection process.

same criteria, an additional 23 articles were included. The process of article selection can be seen in Figure. 1.

Facial nerve palsy

Anatomy of the facial nerve

Facial nerve palsy involves dysfunction of cranial nerve VII, or the facial nerve, which originates from the lower pons and is critical for facial expression, middle ear sensation, taste, salivation, ocular surface protection, and lacrimation. After exiting the brainstem and passing through the parotid gland, the nerve divides into 5 major branches: frontal/temporal, zygomatic, buccal, marginal mandibular, and cervical.[3]

Etiology and the manifestation of facial nerve paralysis/palsy

Bell's palsy, the most common form of facial nerve palsy, is believed to result from idiopathic inflammation, causing nerve compression, ischemia, and demyelination.[5] Other causes of facial paralysis include herpes simplex virus, bacterial otitis media, and Lyme disease, which can lead to significant disabilities such as difficulties in drinking and eating, loss of facial expression, slurred speech, depression, and severe eye complications.[3] Ramsay Hunt syndrome, caused by herpes zoster reactivation, presents with more pain and lower recovery rates compared with Bell's palsy, while Lyme disease can cause bilateral facial nerve dysfunction months after infection.[6]

Treatment of FP

Treatment varies based on the etiology: idiopathic Bell's palsy is treated with corticosteroids,[7] Ramsay Hunt syndrome with steroids and antivirals, and infections with antibiotics. For facial nerve paralysis resulting from trauma or compression, management may involve corticosteroids, surgical interventions like browpexy, botulinum toxin injections, tarsorrhaphy, and more complex reconstructive surgeries such as temporalis tendon transfer or facial nerve grafting. Despite advancements, long-term management of chronic facial nerve palsy remains challenging, with only about 60% of patients achieving excellent cosmetic outcomes.[3]

Current status of facial nerve repair development

Current advancements in facial nerve repair focus on several techniques. Direct suturing is widely used for small peripheral nerve gaps <5 mm) due to its effectiveness in the precise alignment and connection of nerve ends, often yielding favorable outcomes and being preferred by surgeons. However, it is less feasible for larger gaps and can involve excessive manipulation of nerve tissue, potentially leading to additional damage and impaired recovery.[8–10]

Epineural and fascicular suturing for nerve repair are the 2 most widely used techniques. The repairing of the facial nerve is categorized into 3 categories by time: 1) immediate nerve repair within 72 h, which is mainly done by primary neuropathy or interposition nerve grafting after looking into other factors such as the mechanism of injury and local considerations (e.g., soft tissue damage, the extent of nerve damage, hardware, and others); 2) subacute (72 h to a year or one and a half years), so within this time frame, interposition nerve graft is used; and 3) longstanding (beyond 12–18 months), which most of the operative ways are unsuccessful.[11]

Nerve grafting, including autologous, allogeneic, and nerve graft transplantation, is used for larger gaps but requires obtaining nerves from other areas,[12–15] often sensory nerves, which may not fully restore motor function.[16] Nerve transfer, involving the transfer of contralateral nerves like the masseter and hypoglossal muscles to the facial nerve, can provide natural and symmetrical facial expression but cannot fully restore neural function.[17–19] Fibrin glue is an alternative to direct suturing for small gaps, with lower rates of inflammation and easier application for less experienced surgeons.[10,12–20] Drug treatments, such as steroids, dexamethasone, aminoguanidine, and melatonin, show promising results to reduce inflammation and promote nerve regeneration.[21–23] Neurotrophic factors such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and neurotrophin (NT)−3 and NT-4/5 play a crucial role in preserving the microenvironment necessary for nerve fiber regeneration following trauma.[24]

A meta-analysis of 1358 patients with permanent flaccid facial paralysis found that 42.67% of those who underwent direct facial nerve reconstruction, 46.67% through the cross-face nerve suture technique, and 63.11% through the masseteric-facial nerve suture experienced beneficial outcomes. The study also found that 63.89% of patients experienced satisfactory results from facial reanimation using the hypoglossal nerve and 66.43% from facial nerve repair using an interposition graft suture. In total, 778 patients who participated in the trials included in the study received peripheral facial nerve reanimation using the hypoglossal nerve as a cross-motor nerve. Findings showed 54.90% satisfactory results with the traditional hypoglossal-facial nerve suture, 60.53% with the jump-graft approach, and 66.35% with the hemihypoglossal procedure. Overall, 82.35% of patients had excellent outcomes via reanimation using a divided hypoglossal nerve.[25]

Fat grafting and stem cells for regenerative therapy

Recently, regenerative therapy has garnered significant interest due to advancements in genetic engineering and surgical techniques. This field focuses on using stem cells and progenitor cells to repair and regenerate damaged tissues or organs.[26] Stem cells' abilities for infinite self-renewal, differentiation, and various trophic effects position them at the forefront of regenerative medicine.[26,27]

Stem cells are classified by their differentiation capacity (unipotent, multipotent, pluripotent, and totipotent) and origin (embryonic and tissue-derived).[26,27] Embryonic stem cells are pluripotent but

face ethical challenges and risks of teratoma formation.[26] Fetal stem cells are multipotent with fewer ethical restrictions but remain controversial.[26]

Adult stem cells, particularly mesenchymal stem cells (MSCs) from bone marrow and adipose tissue (ADSCs), are highly studied due to their multilineage differentiation, immunomodulation, and regenerative abilities.[26] ADSCs, harvested through liposuction with minimal adverse effects, yield more multipotent cells per milliliter than bone marrow, making them a vital source for regenerative therapy.[26,28] ADSCs can differentiate into endodermal, mesodermal, and ectodermal cell lines, and are suitable for both autologous and allogeneic treatments due to their low immunogenicity.[26,28] Clinical applications of ADSCs span aesthetic, orthopedic, immune system, cardiovascular, craniomaxillofacial, skin and connective tissue, nervous system, and metabolic diseases.[26] They can be injected as cell suspensions or combined with biomaterials to aid tissue regeneration.[29]

Fat grafting and ADSCs for facial paralysis

Animal studies

Several studies were conducted to understand the role of ADSCs in facial nerve regeneration; however, most were conducted in rat models. In general, after making an excision into the facial nerve branch, a conduit made from an artery was inserted into the nerve gap, and epineurium was secured to the adventitia using sutures. Next, the nerve stump at both ends was filled with either culture media, autologous ADSCs, nerve graft, or polyglycolic acid, depending on the allocated groups (see Table 1 for the studies summary).

A study on 8-mm facial nerve branch lesions in a rat model compared autografts in 3 groups: decellularized artery conduits group, decellularized artery and ADSCs group, and nerve autografts group. After 8 weeks of surgery, in contrast to the severed nerves sutured with artery conduits alone, the artery-ADSCs group experienced better regenerative outcomes associated with functional improvement, great axonal development, and improved target reinnervation. Additionally, the number of labeled facial neurons rose, myelinated fiber maturation occurred, and whisking dramatically recovered in transected nerves restored by nerve autografts.[29] Although the result of the decellularized artery and autologous ADSCs group was inferior to the nerve autograft group, it shows an advantageous effect in promoting nerve regeneration. This study poses autologous ADSCs as a choice for facial nerve palsy reconstruction because it facilitates axon regeneration across the nerve lesion and improves its function recovery.[30]

In an in vivo experiment, repairing a 7 mm gap in the rat facial nerve was assessed after a silicone conduit containing undifferentiated ADSCs, developed ADSCs, or Schwann cells (SCs) was implanted and embedded in a collagen gel. After a 13-week regeneration period, a morphometric quantification study of regenerated facial nerves revealed that the ability for nerve regeneration was similar in undifferentiated ADSCs, differentiated ADSCs, and SC groups. Additionally, using an FP grading system, the functional recovery of facial nerve regeneration in the 3 groups was comparable to that of autologous nerve transplant-positive controls.[31]

Kamei et al.[32] experimented with 34 male Lewis rats aged 2 to 8 weeks old. The rats were divided into 5 groups: an autograft, a polyglycolic acid (PGA), a hybrid PGA group with an interposition jump-graft (IPJG) and an ipsilateral great auricular nerve, a PGA tube, a PGA tube containing ADSCs, and a control group that received no therapy.[32] After 13 weeks of operation, all groups showed macroscopic nerve regeneration with myelin sheath and axonal regeneration. The autograft group had a significantly greater mean of 1852 ± 365 regenerated myelinated fibers compared with the other 2 IPJG treatment groups ($P < .01$).[32] The hybrid PGA group had a mean of 320 ± 210, while the PGA group had a mean of 177 ± 78.[32] The hybrid PGA group had considerably thicker myelin (0.68 ± 0.29 μm; $P < .01$) than the PGA group (0.44 ± 0.03 μm), while the autograft group had thicker myelin (0.79 ± 0.03 μm).[32] All groups' compound muscle action potential (CMAP) measurements showed that their muscles contracted upon stimulation. The autograft group had the highest amplitude (4352 ± 1587 μV), which was followed by the control groups (687 ± 490 μV), PGA (1961 ± 445 μV), and hybrid PGA (3222 ± 1779 μV).[32] These findings showed that when nerve con-

Table 1
Summary of animal studies on using adipose-derived stem cells (ADSCs) in facial paralysis repair.

Study	Sample size	Methodology	Main findings
Sun et al.,[30] 2011	40 young female Sprague Dawley rats weighing 100–150 g (aged 5–6 weeks)	In vivo study • Animals were randomly divided into four groups: artery conduit group (1), artery-ADSCs group (2), nerve autograft group (3), and sham-operated group (4). Surgical procedure • A section of the buccal branch was excised from group 1, leaving a nerve gap that was filled with culture media. The epineurium was secured to adventitia using two 11–0 atraumatic sutures, leaving an 8-mm nerve gap after the nerve stump at each end was inserted 1 mm into the decellularized allogeneic artery conduit (1 cm). • In group 2, identical procedures were carried out the same as in group 1 for the conduit insertion, but in addition, a conduit was injected with a solution containing autologous ADSCs in the culture medium. • In group 3, a segment of the corresponding nerve branch was reversed and reimplanted. • In group 4, the corresponding nerve was only exposed and remained intact. Evaluation • Rats were randomly chosen from each group after 8 weeks. After being cut out, the mid-segments of intact or regenerated nerves were treated, and then myelination thickness and density were analyzed using a light microscope.	Regeneration of myelinated nerves • Cross-sections from group 1 revealed reduced regeneration, while sections from groups 2 and 3 showed higher numbers of myelinated fibers, with group 3 exhibiting superior axonal organization. In group 4, the regular distribution of myelinated fibers with homogeneity suggests normal neural anatomy. • Quantitative analysis indicated that groups 2 and 3 had a significantly higher density of myelinated fibers compared with groups 1 and 4. Myelination thickness was significantly greater in groups 4 and 3 compared with group 1. Additionally, group 3 achieved superior outcomes with thicker fibers compared with group 2. Fluorogold-labeled motoneurons number • The number of neurons transmitting axons over the grafts was significantly higher in groups 2, 3, and 4 compared with group 1. Group 3 also had a higher number of FG-labeled neurons compared with group 2. Conclusion • As such, ADSCs did show a benefit in increasing regenerative outcomes of nerves with improvement in function.

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Table 1 (*continued*)

Study	Sample size	Methodology	Main findings
Watanabe et al.,[31] 2017	Eight-week-old male syngeneic Lewis rats	Preparation of ADSC sheets • Adipose tissue from inguinal fat pads was used. • Differentiation of undifferentiated ADSCs (uADSCs) into a Schwann cell (SC) phenotype of differentiated ADSCs (dADSCs) was done. • uADSCs, dADSCs, and SCs were embedded in a silicone conduit in a collagen gel. Surgical procedure • Under a microscope, a gap was formed in the buccal branch of the facial nerve. • Into the space in the buccal branch of the left facial nerve, undifferentiated ADSCs, dADSCs, SCs, and collagen gel alone as a negative control were transplanted. In addition, an autologous nerve graft transplantation was carried out. Evaluation • Pathological examination was done on regenerated nerves and autologous nerves thirteen weeks following transplantation.	Histological examination • When compared with the regenerated nerves in the uADSC, dADSC, and SC groups, the regenerated nerves in the control group were often thinner and had less vascular tissue surrounding the regenerated nerve. The majority of the regenerated nerves were highly organized, with minimal connective scar tissue and rather dense regeneration of axons, as demonstrated using specimens of the middle from the control, uADSC, dADSC, SC, and autologous nerve transplant groups. Regeneration of myelinated nerves • The autologous nerve graft group had the highest number of myelinated fibers, followed by the dADSC, uADSC, and SC groups, all of which had significantly more myelinated fibers than the control group. • Myelin thickness was considerably higher in the autologous nerve graft and dADSC groups compared with the control group. The uADSC and SC groups did not show significant differences in myelin thickness compared with the control group. • The area of regenerated nerve fibers was significantly larger in the autologous nerve graft group, followed by the SC, dADSC, and uADSC groups compared with the control group. The SC group had a significantly larger area of regenerated nerve fibers compared with the uADSC and dADSC groups, indicating a similar potential for nerve regeneration in the latter 2.

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Table 1 (*continued*)

Study	Sample size	Methodology	Main findings
			Facial palsy functional improvement • All rats showed progressive improvement in facial palsy scores (FPSs) after transplantation. After 6 and 13 weeks, the FPS in the uADSC, dADSC, and SC groups increased significantly more than in the control group. The FPS in these groups eventually matched that of the autologous nerve transplant group after 12 weeks. There was no significant difference in FPS among the uASC, dASC, and SC groups over the recovery period. Conclusion • This study has shown that both differentiated and undifferentiated ADSCs can be used as a source of SCs in the therapeutic application of facial nerve regeneration as an alternative to autologous nerve grafts.

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Table 1 (*continued*)

Study	Sample size	Methodology	Main findings
Kamei et al.,[32] 2018	34 two- to eight-week-old male syngeneic Lewis rats	Experimental design • Adipose tissue was collected from the inguinal region. • The rats were divided into 4 groups: the autograft group, the PGA (polyglycolic acid) group, and the hybrid PGA group underwent interpositional jump-graft (IPJG) with an ipsilateral great auricular nerve, PGA tube, and PGA tube containing ADSCs, respectively, and the nontreatment rats (the control group). • Histopathological and physiological assessments were performed at 13 weeks postoperatively. Surgical procedure for preparing a nerve paresis model: • Facial nerve (main trunk; MT), hypoglossal nerve (HN), and greater auricular nerve (GA) were fixed. • Paresis was made by a ligature clip on the facial nerve MT. • IPJG was performed with the ipsilateral GA nerve. • IPJG was performed with a PGA nerve conduit. • IPJG was performed with a hybrid PGA nerve conduit. • A 7-mm nerve bridge was anticipated to develop in the artificial nerve conduit after a 1.5-mm slit was created at both ends for the individual insertion of the hypoglossal and facial nerves in the PGA and hybrid PGA groups. • The face or HN was used as the epineural window for end-to-side neurorrhaphy.	Nerve regeneration observations • Autograft, PGA, and hybrid PGA groups showed macroscopic nerve regeneration at 13 weeks postoperatively. • Toluidine blue-stained specimens revealed myelin sheath and axonal regeneration in the autograft, PGA, and hybrid PGA groups. • Distinct dense nerve regeneration was observed in the autograft group compared with the PGA and hybrid PGA groups. • Electron microscopy revealed myelinated nerve regeneration in the autograft, PGA, and hybrid PGA groups. • The autograft group had a significantly higher mean number of regenerated myelinated fibers compared with the PGA, hybrid PGA, and 2 IPJG treatment groups. • Myelin thickness was significantly higher in the autograft group, followed by the hybrid PGA group, and then the PGA group. • The axon/fiber diameter ratio of the PGA group was significantly higher than that of the autograft and hybrid PGA groups. • Compound muscle action potential (CMAP) measurement revealed muscle contractions after stimulation in all groups. Amplitude was greatest in the autograft group, followed by the hybrid PGA, PGA, and t control groups. Conclusion • These findings showed the effectiveness of IPJG with a hybrid PGA conduit in facial nerve paresis.

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Table 1 (*continued*)

Study	Sample size	Methodology	Main findings
Fujii et al.,[33] 2020	• Eight-week-old male Lewis and GFP transgenic rats • Two rats served as donors of ADSCs and 24 rats underwent cross-facial nerve grafting (CFNG) surgery.	• Preparation of ADSC sheets: ADSCs were obtained from the subcutaneous adipose tissue. • Surgical procedure: Facial paralysis was created by ligating and transecting the main trunk of the left facial nerve. The sciatic nerve was used for (CFNG). • Three groups were established: CFNG alone, CFNG coated with an ADSC suspension, and CFNG wrapped in an ADSC sheet. • CFNG group ($n = 8$): The autologous nerve graft was linked anterogradely to the palsy branch on the left and the healthy mandibular branch on the right. • CFNG + ADSC suspension group ($n = 8$) using a 1-ml syringe at a concentration of 1.5×10^6 cells/ml surrounding the autologous nerve graft right after CFNG and before wound closure. • CFNG wrapped with ADSC sheet group ($n = 8$) underwent CFNG using an autologous nerve graft wrapped in an ADSC sheet, which was created from 1.5×10^6 cells in a 35 mm dish. • Under a microscope, a 9–0 nylon suture was used for all epineural anastomoses. • Evaluation: Nerve regeneration was assessed histologically and physiologically at 13 weeks after transplantation.	Functional recovery • The FPSs were higher in the ADSC sheet group compared with the suspension and CFNG groups at 5 weeks after surgery. • The ADSC sheet group showed a significantly higher CMAP amplitude compared with the suspension and CFNG groups. • No significant difference was observed in CMAP latency or the degree of whisker stimulation among the 3 groups. Myelinated fibers number • The toluidine blue-stained regenerated nerve specimens showed varying numbers of myelinated fibers at the proximal, middle, and distal points: the CFNG group had the lowest, followed by the ADSC sheet group, and the suspension group had the highest. Myelin Thickness • The ADSC sheet group exhibited highly thick myelin in regenerative axons, indicating a potential index of maturation of the regenerative axon. This was higher compared with the suspension and CFNG groups. Conclusion • As such, the CFNG technique with ADSC sheets did show a benefit in promoting axonal outgrowth and decreased reinnervation time of the nerve graft.

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Table 1 (*continued*)

Study	Sample size	Methodology	Main findings
Sun et al.,[34] 2011	60 young female Sprague Dawley rats	• The rats were divided into 6 groups: artery conduit group (1, negative control); artery-ADSCs group (2); artery-dADSCs group (3); artery-SCs group (4); nerve autograft group (5); and sham-operated group (6). • Autologous ADSCs were harvested from rats in groups 2 and 3, and autologous SCs were harvested from rats in group 4. Surgical procedure • Section of the buccal branch nerve was excised distal to the main nerve trunk bifurcation. • A nerve stump was placed into a decellularized artery conduit, secured with two 11–0 atraumatic sutures, creating an 8-mm nerve gap. Cell suspension was injected into each conduit, preventing cell leakage. • Similar procedures were performed in the artery conduit group (1), but Matrigel was used exclusively to fill the artery conduit. • An 8-mm section of the matching nerve branch was inverted and reimplanted in the nerve autograft group (5). • The matching nerve branch was visible but not removed in the group (6) that had sham surgery. EvaluationAfter 8 weeks, the rats were evaluated for their functional movement, CMAP, regeneration of nerve segments, and several labeled motoneurons.	Electrophysiological assessment of CMAPs • The latencies of CMAP recorded in animals in the surgical repair groups (1–5) were higher than those obtained in intact animals (group 6), whereas the peak amplitudes of CMAP and nerve conduction velocity (NCV) values were lower. • Specifically, there was no significant difference in the latency of CMAP between the cell-seeded artery conduit groups 2–4, but there was a significant difference in the peak amplitude of CMAP between each surgical repair group. • Group 4 and Artery-dADSCs group 3 did not substantially differ in their NCV values. However, they both exhibited significantly slower times than those in group 5 and significantly faster times than those in group 2. Nerve segments • The myelinated fiber diameters in groups 3–5 were significantly larger than those in group 1, with no substantial difference between the artery-dADSCs group and group 4, but both were greater than group 2. • While the results in the Artery-dADSCs group (3) and group 4 were not substantially different but were significantly greater than those in group 2, the myelinated fiber diameters in groups 3–5 were significantly larger than those in group 1. • Groups 4 and 5 had considerably thicker myelination compared with group 1, but there was no significant difference in values across the cell-seeded artery conduit groups (2–4). • While the myelinated fiber count in the transected nerves restored using dADSC-seeded artery conduits was abnormally high, the regenerative results related to the maturation of the regenerated fibers were not as good as those obtained with nerve autografts (group 5).

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Table 1 (*continued*)

Study	Sample size	Methodology	Main findings
			Conclusion • As such, dADSC-seeded artery conduits did show a satisfying regenerative outcome of nerves with functional restoration and thus represent an alternative strategy in the reconstruction of facial nerve defects.

duits spanned nerves through end-to-side neurorrhaphy, ADSCs in the conduits encouraged neuron regeneration and the formation of a thicker myelin sheath.[32]

In contrast to the previously mentioned studies, a study in 26 eight-week-old male Lewis rats with facial paralysis divided the rat models into 3 groups: cross-facial nerve grafting (CFNG), CFNG with ADSCs suspension, and CFNG with ADSC sheet. The ADSC sheet group exhibited a significantly higher FP score than the suspension and CFNG groups after surgery, at 10 weeks ($P < .05$) and 5 weeks ($P < .05$). The ADSC sheet group's CMAP amplitude (4.2 ± 1.3 mV) was observed to be substantially higher than that of the suspension (1.7 ± 1.2 mV) and CFNG groups (1.6 ± 0.8 mV; $P < .01$). Between the 3 groups, there was no discernible variation in either the degree of whisker stimulation or CMAP latency. Immunofluorescence staining revealed the presence of green fluorescent protein-positive cells with newly formed CD31-positive blood vessels around the nerve in the ADSC sheet group. The toluidine blue-stained regenerated nerve specimens of the CFNG, suspension, and the ADSC sheet group showed 1700 ± 1018, 3051 ± 1646, and 2736 ± 1120 myelinated fibers at the proximal, middle, and distal points, respectively. In terms of myelin thickness, the ADSC sheet group was found to be significantly higher (0.8 ± 0.5) compared with that of the suspension (0.7 ± 0.3) and CFNG groups (0.6 ± 0.2; $P < .01$). The high engrafting potential of ADSC sheets, facilitated by their maintenance of cell-cell junctions and intermolecular adhesive forces, may have contributed to the improved outcomes in terms of myelinated fiber count and myelin thickness. The faster reinnervation of vibrissal muscles and greater functional recovery observed in the ADSC sheet group compared with the other groups may be linked to the accelerated axonal outgrowth facilitated by ADSCs within the nerve graft.[33]

In a rat model with an 8-mm facial nerve branch lesion, Sun et al.[34,35] examined the impact of a decellularized allogeneic artery conduit containing autologous transdifferentiated ADSCs (dADSCs). The reconstructed transected nerves using dADSC-seeded artery conduits demonstrated satisfactory regenerative results with improvements in morphology and function that were comparable with those obtained with SC-seeded artery conduits—better than those obtained with artery conduits alone or ADSC-seeded artery conduits, but not as good as those obtained with nerve autografts. In addition, many transplanted PKH26-labeled dADSCs were active in axonal regeneration and remyelination while retaining their acquired SC phenotype and myelin sheath-forming ability inside decellularized artery conduits. The combination of autologous dADSCs and decellularized allogeneic artery conduits significantly improved nerve regeneration and functional restoration. As such, this study showed that this method could offer an alternative for reconstructing peripheral facial nerve deficits.

Mechanism of ADSCs in nerve regeneration

Fujimaki et al.[36] reported that dedifferentiated fat (DFAT) cells retained fibroblast squamous morphology and displayed auto-regenerative potential. The DFAT group rat model had more myelinated fibers and greater fiber diameters than the control group. DFAT cells have various advantages, including the fact that they can be acquired without the need for sophisticated cell-selection methods, unlike ADSC or stromal-vascular-fraction (SVF). Other than that, only $\leq$1 g of mature adipocytes is required for the preparation of the cells to minimize the damage to the adipocyte collection site, and a large number of cells can be prepared quickly by conventional methods. Vascular endothelial growth factor, which is known to be released from DFAT cells in excessive quantities by protein array analysis, and the pluripotency of DFAT cells, which may aid in nerve regeneration, could be the causes of the promotion of nerve regeneration in the DFAT group.

ADSCs can differentiate into many lineages, but most importantly, into SC-like cells that guide axon regeneration. ADSCs also provide regenerative potential to healing peripheral nerves by secreting various neurotrophic and angiogenic factors, including glial-derived neurotrophic factor, BDNF, insulin-like growth factor 1, ciliary neurotrophic factor, NGF, and NT-3 and −4. In addition, ADSCs can have a paracrine effect by secreting factors that create a desirable microenvironment for the healing nerve. Additionally, after demyelination has taken place, ADSCs can secrete exosomes, which stimulate the production of myelin basic protein to promote the myelination of injured peripheral nerves.[29] ADSCs may suppress the connective tissue reaction at the coaptation location in some way, allowing a higher number of daughter axons to cross this area. Immunohistochemistry has verified that acetyl-

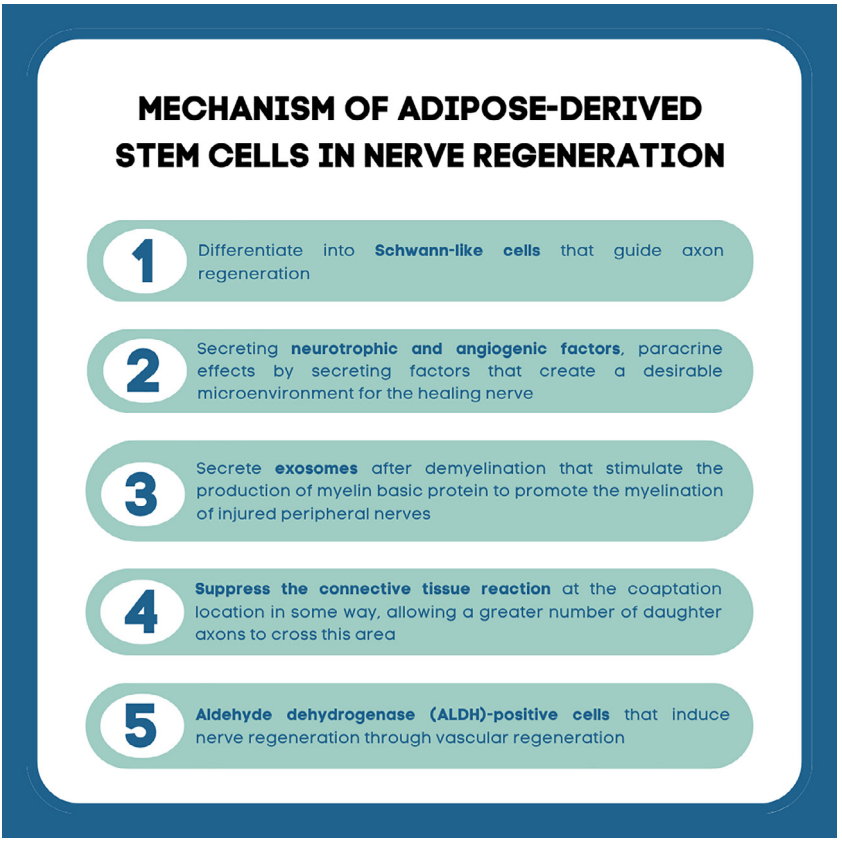

Figure 2. Mechanism of ADSCs in nerve regeneration.

choline is present in the neuromuscular junctions of the mice treated with ADSCs. In addition to being the primary excitatory neurotransmitter in the peripheral nervous system, acetylcholine can stimulate synaptic formation and neurite outgrowth. The growth and differentiation of SCs can be regulated by acetylcholine.[37]

ADSC sources are from SVF, composed of MSCs, pericytes, endothelial cells, smooth muscle cells, lymphocytes, and tissue macrophages. SVF promotes nerve regeneration by transforming the differentiation potential of MSCs into SC-like cells. MSCs in SVF can differentiate into cells with neuronal phenotypes, according to previous in vitro and in vivo studies. Therefore, both differentiated and undifferentiated MSCs can promote axonal regeneration and, eventually, motor performance, in addition to having growth factors. Furthermore, SVF is a cell population consisting of various non-adherent hematopoietic lineage cells that also automatically have aldehyde dehydrogenase (ALDH)-positive cells, a novel marker of hematopoietic stem cells. ALDH-positive cells were hypothesized to induce nerve regeneration through vascular regeneration.[38] The postulated mechanisms of ADSCs in nerve regeneration are summarized in Figure 2.

Human observational studies

There were 2 observational studies in patients with FP. Pappalardo et al.[39] studied the secondary revision technique to improve facial aesthetics in patients with FP using fat grafting as a tissue filler. Thirty of their patients had unilateral FP, and 2 had bilateral FP, consisting of 5 men and 27 women. After initial dynamic reanimation procedures, autologous fat grafting (AFG) was used as a second re-

vision technique to fix facial asymmetry, abnormal contour, and visible muscle pull. Following AFG, postoperative quantitative facial symmetry showed a significant improvement ($P \leq .001$). The average two-dimensional analysis improvement in facial symmetry postoperatively was 10.9%. At the 1-year follow-up, the mean satisfaction scores were 3.31 $\pm$ 0.59 by the patients, 3.06 $\pm$ 0.62 by the surgeon, and 3.16 $\pm$ 0.57 by the independent evaluator. The technique provided aesthetically pleasing results, fewer complications, reduced operating theater time, and improved quality of life for patients. However, AFG faces several challenges, including the need for multiple fixes for graft reabsorption and overcorrection to restore volume. ADSC-enriched fat grafting techniques are receiving increased interest as a means of addressing this limitation. Because ADSCs generate various stem-regenerative cell types, it may be possible to improve the quality and survival of grafts through the collaborative interplay of these cells and the factors they produce.[39]

Siah et al.[40] reported using AFG to achieve periorbital symmetry in patients with FP. The authors significantly improved tear trough visibility ($P < .01$) and lower eyelid–cheek-junction symmetry ($P < .01$) throughout the initial postoperative phase, continuing to improve in the latter parameter into the middle postoperative phase ($P < .01$). However, the variable resorption rate of the grafted fat over time suggested the procedure may require repeating in some patients. Furthermore, although more research is needed to confirm this notion, the authors speculate that the beneficial properties of autologous fat might help relieve the skin contraction of the upper eyelid and might even have some effect on the levator palpebrae's thixotropy.[40]

Future perspectives and conclusions

This review was done to achieve a better insight into the use of ADSCs to manage FP. Although a limited number of articles are based on human clinical cases, the rest of the literature on rat models consistently confirms the potential of ADSCs in promoting nerve regeneration and functional recovery. ADSCs improve regeneration by boosting functional recovery, encouraging substantial axonal development, increasing the number of myelinated fibers, thickening the myelin sheath, and enabling improved target reinnervation. ADSCs offer a potential choice for the reconstruction of facial nerve palsy, even though the outcomes might not be as good as nerve autografts. More experiments and clinical trials on humans are needed to demonstrate the functional benefit of ADSCs from fat grafting in managing facial paralysis.

Conflict of interests

The authors declare no conflict of interest.

Funding

None.

Ethics approval and consent to participate

Not applicable.

Data availability statement

Data sharing does not apply to this article as no datasets were generated or analyzed during the current study.

Acknowledgments

We thank IJCR Central for providing us with research knowledge, learning, and the opportunity to participate in research.

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