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## Stem cells and related factors involved in facial nerve function regeneration

### Komórki macierzyste i czynniki wpływające na regenerację funkcji nerwu twarzowego

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#### Summary

The facial nerve (VII) is one of the most important cranial nerves for head and neck surgeons. Its function is closely related to facial expressions that are individual for every person. After its injury or palsy, its functions can be either impaired or absent. Because of the presence of motor, sensory and parasympathetic fibers, the biology of its repair and function restoration depends on many factors. In order to achieve good outcome, many different therapies can be performed in order to restore as much of the nerve function as possible. When rehabilitation and physiotherapy are not sufficient, additional surgical procedures and therapies are taken into serious consideration. The final outcome of many of them is discussable, depending on nerve damage etiology. Stem cells in facial nerve repair are used, but long-term outcomes and results are still not fully known. In order to understand this therapeutic approach, clinicians and surgeons should understand the immunobiology of nerve repair and regeneration. In this review, potential stem cell usage in facial nerve regeneration procedures is discussed.

**Key words:** facial nerve repair • stem cells • nerve regeneration • immunobiology • nerve fibers

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## INTRODUCTION

The facial nerve is the seventh (VII) cranial nerve. In the cranium it arises from the brainstem between the pons and the medulla and divides itself into main intracranial branches (greater petrosal nerve, stapedius nerve and chorda tympani). After it arises from the stylomastoid foramen at the temporal bone, it divides further into extracranial branches (posterior auricular nerve, nervous branch of the posterior digastric and stylohyoid muscles). The major motor facial branches of the facial nerve form a plexus in the parotid gland mass, consisting of 5 main motor branches: temporal, zygomatic, buccal, marginal mandibular and cervical. Any surgery performed in the head and neck region should include anatomical localization of the nerve branches. In any case of nerve injury or palsy, different steps should be made to minimize its damage and regain as much function as possible [16].

Stem cells (SC) are a type of special undifferentiated cells that have a unique ability to differentiate after mitosis into any specialized cells. There are two types of most known stem cells, such as embryonic stem cells (ESC) and adult stem cells (ASC). Stem cells can be used from autologous transfers from different parts of the human body. Bone marrow, adipose tissues, blood and umbilical cord blood can be used as donor sites for stem cells. Both adults and children can deliver SC of various origins, but mostly nowadays adults are the main participating group in this type of therapy. In some cases fetal stem cells (FSC) could be used, but they are rarely used in nerve repair because of the technical difficulty to obtain them [17].

Many studies are taking place worldwide in order to discover various therapies that could be used in tackling some human diseases such as leukemia, cancers, sclerosis or Parkinson's disease. The main syndromes and diseases that target various nerve fibers in the human body still remain incurable and late outcomes of known therapies remain unsatisfactory. Perhaps SC usage in peripheral and central nerve repair would improve patients' quality of life. The CNS (central nervous system) suffering from degenerative nerve disorders such as Alzheimer's or Parkinson's is nowadays considered as impossible to fully repair.

Facial nerve regeneration with usage of stem cells has been used with different clinical outcomes. Yet so far long-term clinical outcomes are not fully known. Laboratory studies have their limitations; however, some results are promising and can influence the degree of facial nerve repair. Because of the presence of sensory, motor and parasympathetic fibers, studies should indicate the type of nerve repair for the selected branch of the facial nerve. Secondly a homogeneous study group should be carefully selected to assess similar enough clinical parameters of nerve damage. Because of neurons' ability to regrow and self-repair, it is important to remember that the peripheral nervous system (PNS) has better repair ability than the central nervous system (CNS). Axon formation along the injury site requires additional autologous nerve grafting

for improved function restoration. Nowadays three direct approaches for facial nerve repair are used: direct repair, nerve substitution techniques and cable nerve grafting (end-to-end nerve anastomosis). Because of very complicated facial nerve function and regeneration patterns, every clinician should be aware that not only preserved numbers of nervous fibers are essential to achieve better therapeutic outcome. Also type of damage that the nerve has suffered from is important. After facial nerve damage, in its direct proximity different types of immune cells and molecules gather in order to repair the nerve. The degree of facial nerve palsy, injury or lack of function is related to various etiological factors and their influence on the nerve itself in different places of its localization. Tumors of the temporal and auricular region along with temporomandibular joint and parotid gland malformations are the most common ones. Inflammatory and viral infections can be very significant factors related to facial nerve palsy. Also injuries to the facial skeleton and temporal bone can lead to lack of function of the facial nerve. In all etiological cases, pathways of facial nerve repair might be different, depending on the damage and the time that has elapsed since the incident.

Because of injury or palsy of the facial nerve, the side of the face supported by different facial nerve motor fibers can be affected. Bell's palsy and viral infections are still among the most common ones. A different diagnostic approach can be used in a facial paralysis caused by herpes simplex virus type 1. A rat study performed by Mao et al. using daily methylprednisolone sodium succinate (MPSS) or with combined administration of MPSS and glucocorticoid receptor blocker (RU486) injections suggests that this therapy could be an alternative therapy in viral infections, because of weakening the damage to the nerve system [14].

Because of the proximity of nerve branches to facial anatomical structures, the first symptoms that occur might influence the proper diagnosis. Ptosis, the Bell phenomenon, lack of taste sensation, inability to wrinkle the forehead and others should be symptoms characteristic for different facial nerve branches' palsy or injury. Depending on the number of nerve branches affected by causative agents, different therapeutic methods could be used. In cases of compression injury to the facial nerve, some authors confirm that usage of fibroblast growth factor (bFGF) could improve its regeneration [14]. Injuries affecting larger parts of nerve length are associated with chemical and temperature factors and have less positive therapeutic outcomes than other types of injuries. Nerve fibers, axon accumulation and selected cell interactions between cell-glial, cell-axon, T-cells and glial-Schwann cells are important in enhancing nerve regeneration and future function improvement. When any phase of nerve repair is blocked or insufficient, final outcomes might result in lack of nerve function.

Activity and proliferation of Schwann cells (SCs) are the most important factors in promoting peripheral nerve

regeneration. Increasing cell adhesion molecules (CAMs), such as N-CAM, Ng-CAM/L1, N-cadherin, and L2/HNK-1, influences neurotrophic agents. Overall nerve repair and axonal regeneration are related to adequate enhancement of the growth potential [14,25]. On the other hand, nerve self-recovery potential depends greatly on the damage and its etiology. Cyclic adenosine monophosphate (cyclic-AMP; cAMP) influences proper endogenous nerve cell repair, because of its importance in signaling pathways and SCs' differentiation and proliferation towards damaged axon fibers. The role and activity of activation of CREB and the inhibition of cytoskeletal inhibitors by PKA might be some sources of potential nerve growth stimulation [9,16]. cAMP activity in axonal outgrowth is very important. Some special nerve signaling cascades, such as PI3K/Akt and Ras/ERK, have a positive effect on neuronal survival during its regeneration process. Some therapeutic agents, such as erythropoietin, tacrolimus, acetyl-L-carnitine, n-acetylcysteine and geldanamycin, have a good effect on nerve regeneration function because of their influence on this signaling pathway [3].

Both T cells and cytokines along with molecular signaling pathways take part in facial regeneration and repair. Glial cell activation is an important factor in an adequate pathway of nerve response. A special part in nerve regeneration is achieved by increased expression of NGF (nerve growth factor), a neurotrophin present more often after nerve injury. One of its effects focuses on function of cholinergic neurons in the CNS. Additionally, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) regeneration function is supported by glial cell line-derived neurotrophic factor (GDNF) and ciliary neurotrophic factor (CNTF). In the case of nerve injury, vascular endothelial growth factor (VEGF) influences axonal outgrowth and neuronal survival with flk-1 receptor increasing nerve microcirculation and improving the condition of myelinated nerve fibers [15]. On the other hand, activation of Schwann cells proliferating along nerve fibers enhances myelination and motor function. Schwann cells (SCs) and the other sub-divisions of glial cells from the PNS are valuable transplantable nerve cells for regeneration and influence on axon re-growing interactions.

In cell signaling pathway, special cell signaling has to be present. T-cells affect cytokines so that signaling pathways (cell death, cell adhesion) can influence direct facial nerve repair and regeneration. In order to understand the nerve repair process the relation between the nervous system and nerve anatomy should be known [6].

Transcription factors such as c-Jun, activating transcription factor 3, cAMP response element binding protein, signal transducer, and activator of transcription-3, CCAAT enhancer binding proteins  $\beta$  and  $\delta$ , Oct-6, Sox11, p53, nuclear factor kappa-light-chain-enhancer of activated B cell, and ELK3 have a significant role in peripheral nerve regeneration. Activator of transcription-3 (STAT family transcription factors - STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6) regulates CNS and PNS cell

growth, regulation, inflammation and embryological development. STATs are activated after injury. Selected cytokines and growth factors such as IL-6, LIF, CNTF, G-CSF, G-CSFR, EPO, EGF, IGF-1, NGF withdrawal, BDNF, free radicals, excitatory neurotransmitters and other inflammatory mediators activate the signaling pathway in nerve injury [6,15,16,19,27]. Schwann cells along with direct proximity of injured nerve fibers can accumulate along axons which are related to the regeneration process. Antigen-presenting cells (APC) and T cells with CD4 prevalence affect nerve fibers [7].

Nerve growth factor (NGF) promotes peripheral regeneration of nerve fibers. A study by Li et al. indicated that Let-7 microRNAs (miRNAs) reduce primary SC migration and NGF protein translation. Use of inhibitor of Let-7 allowed an increase of NGF secretion and SC proliferation on injured nerve. This in vivo study indicates a new therapeutic method for peripheral nerve injuries [13].

## STEM CELLS

Stem cell (SC) therapy and its application in different parts of the human body is still a focus of studies worldwide. In this review paper we present a brief report about use of stem cells in facial nerve regeneration. Each nerve consists of connective tissue layers called the endoneurium, perineurium and epineurium. Regenerative therapies describing axon repair techniques are focused mostly on restoration of the entire axon structure, but the degree of restoration of each layer might vary greatly depending on the techniques and approach used. It is important to keep in mind that because of many factors involved in facial nerve function and its possible injury or palsy, all clinicians should be aware that surgical reconstruction techniques including various nerve grafting methods have different rates of success. Perhaps a combined surgical approach using stem cells could improve the final functional outcome and result in more accurate treatment. The use of other materials combined together with SC can improve the singular effect of SC used alone.

Use of stem cells without a proper pathway of neurotrophic factors (NF) will be insufficient for nerve fiber regeneration. Protein ligands and tyrosine kinase receptors related to NF can promote growth and survival of different axon fibers. Activation of genes and molecules involved in growth, proliferation and transport in neurons is related to NF occurrence. The three most important NF that take place in adequate cell signaling in nerve regeneration are the nerve growth factor (NGF), ciliary neurotrophic factor (CNTF) and insulin-like growth factors (IGFs). IGF-1 and IGF-2 can influence the axon regeneration ratio [4,19].

Immunobiological studies performed for understanding nerve repair and regeneration patterns are very valuable. Additional information noted on cell behavior, motor function and axon repair indicates some new cell functions. Peripheral nerve regeneration can be easily incre-



ased or made more accurate after using additional cells, proteins, growth factors and others.

Quality and efficiency of motor nerve function are related to the degree of nerve injury and its regeneration process. Morphogenetic protein-2 (BMP-2) expression and occurrence in studies on rabbits were investigated with in situ hybridization of facial nuclei. After nerve injury and use of BMP-2, nerve fiber degeneration was smaller. This might be related to a neurotrophic factor inducing nerve regeneration [26].

After nerve injury, axon fibers and reactive microglia promote microgliosis present in the CNS. Active microglia influence nerve repair regulation. Ladeby et al. investigated its acute activation phase in the dentate gyrus following transection of the entorhino-dentate perforant path. The authors concluded that the stem cell marker CD34 was upregulated during the early stage of activated microglia more than in infiltrating bone marrow-derived cells. The study showed that two fractions of CD34(+) and CD34(-) with Mac-1(+) have different effects on microglia. CD34(+)Mac-1(+) had more self-renewal activity while CD34(-)Mac-1(+) because of its mixed fractions proliferative function is still unknown [12].

It is well known that SCs are used in different therapeutic procedures. In facial nerve repair and regeneration different variations of SC with or without other additional materials are used. Adult stem cells (ASC) have more advantages than fetal and embryonic stem cells (FSC, ESC). ASCs present in bone marrow contain several types of SC such as hematopoietic stem cells (HSC) and BMSC (bone marrow stem cells, mesenchymal stem cells). Bone marrow stem cells (BMSC) with their sub divisions dBMSC and uBMSC are known. Neural stem cells (NSCs), adipose-derived stem cells (A-DSCs), mesenchymal and mesenchymal stromal stem cells (MSCs), ectomesenchymal stem cells (EMSCs) and different types of SC are used in nerve regeneration techniques.

BMSCs are the most common ones used in therapies. Easily accessible bone marrow is one of the most commonly used SC. The aim of the study performed by Aggarwal et al. was to evaluate mononuclear bone marrow stem cells (BMSCs) in patients after posttraumatic facial nerve paralysis. Cells were applied and their impact on the facial nerve was measured at 3 and 6 months after surgery using the House-Brackmann grading system. The electroneurography (ENoG) response was improved and both eye and mouth closure was statistically more efficient, and in 85% of all patients the results were far better than during standard surgical approaches [1]. Improving expressions and balance of the face is the most desirable effect of therapy. Bone marrow stem cells are an alternative approach for therapy in facial nerve axonal function loss. They can be divided into subgroups, such as uBMSC (undifferentiated BMSC) and dBMSC (Schwann-like cells differentiated from BMSC). Facial nerve regeneration in a rat model study performed by Salomone et al. suggests that dBMSC and

uBMSC had a positive effect on nerve regeneration in the mandibular branch of the facial nerve [16]. Autografting with use of bone marrow stem cells (BMSC) combined with polyglycolic acid tube (PGAT) in rat facial nerves was studied by Costa et al. The rat mandibular branch of the facial nerve was repaired using different approaches and use of autografting, PGAT, matrix membrane, BMSC and Schwann-like cells. The authors concluded that improved facial nerve repair was seen after use of BMSC with PGAT but use of Schwann-like cells also has a positive influence on nerve repair [5]. Also an increased amount of VEGF and blood vessel proliferation within BMSC improves nerve regeneration faster.

Neural stem cells (NSCs) usage in nerve repair and regeneration is still the focus of many studies. NSCs have a special ability to differentiate into neurons, astrocytes and oligodendrocytes. Rabbit studies presented by Zhang et al. with use of NSCs and collagen-neurotrophin revealed increased repair potential. In the ends of the transected facial nerve hyaluronic acid (HA)-collagen composite with neurotrophin-3 and BrdU-labeled NSCs were used. The authors confirmed that combined NSCs embedded in HA-collagen biomaterial could improve peripheral nerve repair. Collagen itself increases nerve reinnervation. The bridging method of nerve repair with use of stem cells is an alternative technique with an increased success rate [28]. Shi et al. used a biodegradable poly-DL-lactide-co-glycolide (PLGA) nerve conduit (NC) filled with NSCs in rats after transection of the facial nerve and coverage of the nerve gap. Later nerve function until the 12<sup>th</sup> week was assessed with electrophysiological testing, and morphometric analysis of axons. The authors concluded that transplanted NSCs helped to increase nerve action potential and amplitude [21]. Using neural cells from other donor sub-sites might have resulted in decreased clinical value. Because of that, Sasaki et al. performed a study using a degradable poly-DL-lactide-co-glycolide (PLGA) tube containing DPCs (dental pulp stem cells). The authors concluded that after filling the gap in the facial nerve its repair was increased, which confirms in vivo studies [18]. Neural stem cells (NSCs) can be an alternative for other stem cells, because of their relation with other nerve structures. They have a natural ability to mature into neurons and even Schwann-like cells. Some authors claim that because of their primitiveness and many donor sites they should be used carefully.

Guo and Dong performed a study on 36 rabbits (*Oryctolagus cuniculus*). A gap of 10 mm facial nerve defect was made in order to observe and evaluate the use of neural stem cells (NSCs). Direct application of NSCs as seed cells on peripheral nerve could be a very good approach to repair nerve defects [10]. Another study involving NSCs and use of a biodegradable nerve conduit (NC) filled with NSCs overexpressing glia-derived neurotrophic factor (GDNF) was performed to measure the accuracy of the repair process. The authors concluded that the combined NSCs-GDNF group has a better effect on axons and their regeneration than NSCs used alone; however, the results were not statistically significant [22].

Ghoreishian et al. performed a study using adipose-derived stem cells in Gore-Tex tubes in order to enhance facial nerve repair. Cells were extracted from autologous adipose tissues from mongrel dogs. After cutting a gap of 7 mm in the facial nerve, an expanded polytetrafluoroethylene tube filled with undifferentiated adipose-derived stem cells was placed and encapsulated in alginate hydrogel. After use of Gore-Tex tubes neural repair was increased, with a more accurate neural functional response [8]. Still further studies need to evaluate these findings and describe how different tissues and cells react. Similar studies were performed by Huang et al. but without Gore-Tex tubes in infant piglets. Quite similar conclusions were reached [11]. Sun et al. used a decellularized allogeneic artery filled with autologous trans-differentiated adipose-derived stem cells (dADSCs) on an 8-mm facial nerve branch lesion in a rat model. The authors concluded that the results were quite similar to those which can be achieved after using Schwann cell (SC)-seeded artery conduits. A valuable aspect of this study is the novel approach to peripheral facial nerve defect reconstruction [23]. The same authors performed a similar study to evaluate outcomes of this technique. According to Sun et al., combined axonal growth with reinnervation and improved function were achieved, which seems to be a more favorable reconstructive approach [24].

Autologous mesenchymal stem cell transplantation could be useful but has its limitations. Caylan et al. stated that use of stem cells in peripheral facial nerve damage can improve the patient's quality of life (QOL) and nerve function [2].

Use of bone marrow mesenchymal stem cells (MSCs) and transdifferentiated Schwann-like MSCs (tMSCs) and their ability to restore nerve function are still under review. A rabbit study after transection of 10 mm of the buccal branch of the facial nerve was carried out by Wang et al. At 4, 8 and 16 weeks post-surgery rabbits were sacrificed and functional, immunohistochemical, and morphological tests were performed. It was found that Schwann-like MSCs with the used autogenous vein graft had better regeneration patterns along with enhanced remyelination [25].

A valuable study performed by Satar et al. compared the expression of myelin-associated glycoprotein (MAG) in rats after transection of the buccal branch of the facial nerve. Different expressions of myelin basic protein (MBP), neural cell adhesion molecule (NCAM)-1, matrix metalloproteinase (MMP)-1A, tissue inhibitor of metalloproteinase (TIMP)-1, TIMP-1/MMP-1A ratio, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), neurotrophin (NT)-3, NT-4, glial cell-derived neurotrophic factor (GDNF), leukemia inhibitory factor (LIF), basic fibroblast growth factor (FGF)-2, insulin-like growth factor (IGF)-1, platelet-derived growth factor (PDGF)- $\alpha$  and transforming growth factor (TGF)- $\beta$ 1 in anastomosed facial nerves that had been treated with or without mesenchymal stromal cells

(MSC) were studied. Yet this complete study brings a new light to the therapy. MSC increased expression of CNTF, PDGF- $\alpha$ , LIF, TGF- $\beta$ 1, BDNF and NT-3, while MAG, NCAM-1, MMP-1A and FGF-2 require further investigation. The authors concluded that regeneration, nerve function, apoptotic activity and further effects of nerve regeneration still require further studies [19].

Mesenchymal stromal cells (MSCs) are able to transdifferentiate into Schwann cells. Satar et al. performed a study on 15 rats after transecting the buccal branch of the facial nerve and used MSCs and evaluated the results in selected time-frames. Histological findings conclude that MSCs used in nerve repair had a significant prevalence on repair and axonal condition [20]. MSCs usage in nerve repair and axon regeneration was also studied by Cho et al. 24 albino guinea pigs were transected and both platelet-rich plasma (PRP) and nMSCs were used. The authors concluded that both factors used together had an increased effect on nerve repair and regeneration. Results were more accurate than other mentioned techniques [4]. An additional technique might involve nerve entubulation. Transected facial nerve filled with isogenic bone marrow-derived mesenchymal stem cells (BM-MSCs) in collagen resulted in lesser function restoration. Grosheya et al. concluded that this method is not good enough for facial nerve repair [9].

Stem cells from dental pulp and periodontium can also be used in order to improve facial nerve function. Wen et al. investigated ectomesenchymal stem cells (EMSCs), derived from the cranial neural crest in rats. The selected p75 neurotrophin receptor (p75NTR) identified the p75(+) EMSCs. It could be a useful diagnostic tool [27].

Other studies worldwide indicate that the method and number of cells delivered in the direct proximity of nerve injury are discussable and different final clinical outcomes are observed. It seems that microinjections directly to the nerve proximity with  $10^6$  or more cells might be a valuable alternative method to cell transplantation [17].

Stem cells are a great alternative to standard surgery, which has its own limitations and complications [15]. Sardesai and Moe in their recent review confirmed our findings and thoughts that facial paralysis and nerve injury repair is still a topic of special interest of many clinicians. Novel approaches and studies are promising. Some special transplanted SC expressing S-100 (a Schwann cell marker) protein are able to differentiate into neural cells and increase myelination. A role of the P311 activation gene and its relation with the nerve regeneration process should be examined further and perhaps a combined gene-stem cell therapy would increase the nerve repair success rate. Experimental medicine and stem cell therapies indicate that there is still a lot to do in order to enhance this branch of medicine [17].

Application of stem cells in peripheral facial nerve injury is promising. Different types of SC delivered locally were



used in the presented studies. So far no late clinical and nerve function outcomes are either known or described. Perhaps achieving full axon regeneration and function restoration will also correlate with the change of nerve fibers structure.

## CONCLUSIONS

The House-Brackmann facial nerve damage scale, electroneurography and facial movement scale can be very useful in describing facial nerve injury and the potential repair and regeneration stage in facial nerve palsy or injury. Many therapeutic outcomes and regeneration techniques are known, but none of them fully recovers nerve function. Use of stem cells in nerve regeneration and their unique ability to differentiate into different cell types might increase the therapeutic success rate. Additionally used materials and biomaterials can increase the success rate. Perhaps a biodegradable conduit filled with SC and cable nerve grafting could be the future for nerve regeneration. Stem cells used at different stages in facial

nerve injury are used to improve the motor function and patients' facial expressions. From our point of view, stem cells are the future of medicine, not only because they have many donor sites, but also because of their ability to differentiate into other types of cells, in this case in neural cells to improve axon function.

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