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An Overview on Nanofat in Peripheral Nerve Surgery

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Abstract:

Background: Peripheral nerve injuries remain a major clinical challenge because of the limited regenerative capacity and incomplete functional recovery after surgical repair. Adipose-derived stem cells (ADSCs) have demonstrated promising regenerative and neurotrophic effects through differentiation into Schwann-like cells, secretion of growth factors, promotion of angiogenesis, and modulation of the local microenvironment. Nanofat, a mechanically emulsified adipose tissue product rich in ADSCs and regenerative mediators, has recently emerged as a minimally invasive regenerative technique with potential applications in peripheral nerve surgery. Experimental studies have shown that adipose tissue derivatives can enhance axonal regeneration, improve myelination, reduce scar formation, and support functional recovery in various models of nerve injury. However, the role of nanofat in peripheral nerve regeneration remains under investigation, and further experimental validation is needed to clarify its therapeutic efficacy and mechanisms of action.

Keywords: Nanofat; Peripheral nerve injury; Adipose-derived stem cells; Nerve regeneration; Schwann cells; Regenerative medicine; Fat grafting; Peripheral nerve surgery.

Introduction:

The following injury to a peripheral nerve, the distal stump will undergo Wallerian degeneration, while the proximal stump will exhibit central chromatolysis. The myelinating cells of the PNS, Schwann cells, provide support to injured nerves attempting to regenerate via axonal sprouts and reinnervate distal structures **(1)**.

Adipose tissue is a complex cellular organ composed of adipocytes, various immune cells, endothelial cells forming blood and lymphatic vessels, and a population of stem and stromal cells. Together, these cell types support the tissue's multiple roles, including its function as an endocrine organ, a reservoir for energy storage, and a central regulator of systemic energy metabolism. Adipocytes make up the majority of the tissue and are the primary sites of fat storage in the body. Notably, adipose tissue exhibits remarkable plasticity, with the capacity to expand or contract substantially within the same individual over time (**2**).

Among the stem and stromal populations within adipose tissue are adipose-derived stem cells (ADSCs), which contribute not only to tissue homeostasis but also to regenerative processes. These cells are capable of differentiating into multiple lineages (**3**), most notably into Schwann-like cells, which play an important role in guiding axonal regeneration (**4**).

However, Predifferentiation of ADSCs into glial or neuronal phenotypes is not essential for achieving significant peripheral nerve regeneration. Several experimental studies have demonstrated that undifferentiated ADSCs (uADSCs) can enhance nerve repair through different mechanisms, including improvements in functional recovery, histomorphological parameters, or both **(5).**

Despite these promising findings, the exact mechanism of action remains unclear. It is still uncertain whether uADSCs differentiate in vivo and directly contribute to tissue regeneration or instead exert their therapeutic effects by modulating endogenous cells and releasing neurotrophic factors that promote nerve repair (**6**).

Recent research suggests that uADSCs might function mainly as supporting components in nerve repair structures. uADSCs were still able to achieve functional recovery comparable to differentiated adipose-derived stem cells (dADSCs in terms of sciatic functional index and nerve conduction velocity **(7)**, even though Schwann cell-like differentiated ADSCs (dADSCs) demonstrate superior capacity to promote nerve regeneration through classical Schwann cell-mediated mechanisms **(8)**.

ADSCs additionally supply regenerative potential to healing peripheral nerves by secreting various neurotrophic and angiogenic factors, including brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF), insulin-like growth factor 1, nerve growth factor (NGF), and neurotrophin (NT)-3 and -4 **(9)**.

The paracrine effect of ADSCs on nerve regeneration has been evaluated in vivo and the factors secreted create a desirable microenvironment for the healing nerve. Enhancing the paracrine effect of ADSCs by controlling the release of neurotrophic factors such as BDNF or providing greater surface area for ADSCs has previously augmented ADSCs, and, on the contrary, the paracrine effect of ADSCs may be inhibited by materials introduced into the microenvironment that inhibit the dissemination of factors **(10)**.

Schwann-like cells have been shown to migrate to sites of injury beyond the distal nerve segment, where they release nerve growth factors to promote nerve growth **(9)**. Molecules involved in Schwann cell morphological changes in response to nerve injury, such as c-Jun, allow for axoglial interactions and regeneration of axon tracts or bridges **(11)**.

ADSCs can also secrete exosomes, which augment the production of myelin basic protein to increase the myelination of damaged peripheral nerves after demyelination has occurred. The biology of adipose tissue, mostly due to the presence of ADSCs, disposes of adipose tissue to be used in surgical procedures to promote healing at the site of injury **(12)**.

Derivatives of adipose tissue include macrofat, microfat, nanofat, and microvascular fragments depending on the manipulation of the tissue. Macrofat and microfat are classically utilized as a filling biomaterial for soft tissue defects and injury, while nanofat is more often used in tissue remodeling because of its injectability and regenerative potential **(13)**.

Conveniently, ADSCs can be applied as cell suspension injections or in combination with various biomaterials to assist in tissue regeneration **(13)**. Autogenous fat grafting is a method of delivering ADSCs without having to process the tissue, which avoids the FDA regulations that are placed on purified cells from adipose tissue **(14)**. In this manner, fat grafting is an accessible method that also keeps costs low, is minimally invasive, and does not pose the risk of an immune response **(15)**.

Adipose Cells Enhance the Regeneration of Nerves

Crush Injury Studies

Crush injury studies are employed to reproduce nerve damage such as acute neuropathy. In these studies, a clamp is placed onto a nerve to provide pressure to, or ―crush‖, the nerve. The nerve experiences damage directly from the pressure and from the induced ischemia due to the comparatively smaller pressure of capillary perfusion relative to the externally applied pressure **(16)**.

Animal experiment results presented in the literature support the continued exploration of ADSC in addition to crushing nerve injuries. A study on peroneal nerves in mice found that crush injuries treated with ADSCs in Matrigel exhibited greater functional recovery compared to injuries not treated with ADSCs as early as 2 days after surgery, as well as at 10 days **(17)**.

In a crush injury study of 40 rats, white adipose tissue flap had positive effects both functionally and histologically on nerve regeneration. Groups treated with adipose tissue demonstrated significantly greater recovery maximum isometric tetanic force than untreated groups, indicating better functional recovery. Adipose-tissue-treated nerves also had a 13% increase in axon number ($p < 0.05$) and a 31% increase in myelin thickness/axon width ratio ($p < 0.001$), indicating better histological recovery **(18)**.

Another study injected ADSCs distal to the site of rat sciatic nerve 14-day crush injuries, which resulted in less muscle atrophy as well as better functional and anatomical outcomes than the group that had the compression clip relieved after 14 days with no subsequent epineural ADSC injection **(16)**.

Quality of Regeneration

Regeneration of the nerve is important, but it is also important to limit scarring during the healing process. Perineural adherences can derail proper healing after peripheral nerve surgery and result in persistent pain. ADSCs can prevent damage to the nerve while facilitating nerve regeneration **(1)**.

In a study of sciatic nerve surgical injury in mice, fat grafting reduced scar tissue development around the healing nerves. Thus, adipose tissue not only can mechanically protect nerves during the healing process by acting as a barrier but can also act as a biological compound with pro-regenerative properties **(19)**.

Recently, ADSCs have been shown to constitutively produce exosomes that contain neural growth factor transcripts **(20)**. The exosomes released by ADSCs create desirable microenvironmental conditions to contribute to neurite regeneration via axon outgrowth. Schwann cells from nerve injury sites cocultured in vitro with ADSCs with varying levels of ADSC-derived exosomes demonstrated that increased exosome levels correlated with decreased apoptosis and increased proliferation of Schwann cells **(21)**.

Preventing scar tissue formation, which decreases peri-neural adherences, is clinically important, especially when considering hand surgery. Carpal tunnel release and Dupuytren's contracture surgery both rely on the unstressed gliding of the nerves and tendons for surgical success. Preventing adherences would facilitate greater surgical success and allow for more smooth hand movements. In a study evaluating the capability of muscarinic receptors to modulate the nerve growth factor production of rat ADSCs, M2 receptor stimulation reduced apoptotic factor proNGF-B, which demonstrates that acetylcholine promotes the maturation of nerve growth factor (NGF) **(22)**.

There are likely other molecules in addition to acetylcholine that can be targeted to improve the regenerative properties of ADSCs. ADSC-derived exosomes have experimentally been manipulated to carry more NT-3 mRNA, which improved nerve recovery in rat models after two weeks **(23)**.

Hypoxic expansion has also been shown to increase the neuronal differentiation potential of ADSCs in vitro **(24)**.

Additionally, theorized but not yet clinically evaluated, β_2 receptor stimulation of peripheral nerves leads to an increase in cAMP, which is a molecule that has been associated with Schwann cell proliferation **(25)**.

Animal Studies Utilizing Fat in Various Types of Peripheral Surgery

Nerve Repair

Nerve primary tensionless repair is indicated after acute transection of a nerve. For nerve transections that can be repaired without undue tension, the gold standard is direct epineural coaptation with micro sutures, though tissue adhesives may be used in place of or in adjunct to micro sutures. To avoid unwanted tension, end-to-end neurorrhaphy should only be used in humans if the nerve gap is 1 cm or less **(26)**.

Following an injury to a peripheral nerve, Schwann cells are activated to enter a repair program state to perform functions including the activation of negative regulators of myelination to demyelinate the damaged nerve, modulation of genes to promote neuron survival and axonal regrowth, and remyelination of the regenerated axon **(11)**. Though Schwann cells naturally act to promote peripheral nerve regeneration and healing, natural repair without surgical manipulation has numerous obstacles. Schwann cells modulate genes only transiently, so, as time passes, the Schwann cells provide less assistance to the nerve, whether it heals properly or not **(27)**.

Regenerating axons are guided to their targets but sometimes fail to enter the correct endoneurial tube and do not successfully reinnervate the target organ. Surgical coaptation of the transected nerve ends can help to prevent errors in reinnervation but still requires guidance from the Schwann cells to heal the nerve after coaptation.

Even then, only around 10% of axons reach the intended target muscle or organ after surgical repair following transaction **(28)**.

Nerve transections are associated with poor outcomes even after repair, especially when the distance between the nerve and the target is long or when the repair is performed after a delayed period **(29)**. Consequently, only transactions that can be repaired without undue tension should be attempted without graft and repairs should take place as soon as possible. Providing extra materials to guide the regenerating axons, such as ADSCs present in adipose tissue that can transform into Schwann-like cells, could be of benefit to nerves repaired by direct coaptation with suture **(1)**.

A study of rat sciatic nerve injury managed to map cell ADSC migration in vivo. After nerve injury, an approximation of the stumps with nerve sutures was combined with ADSC injection, where ADSCs were tracked for 14 days. The experiment confirmed that ADSCs migrated in vivo to the distal section of the nerve injury from the proximal injection site within two weeks, likely guided by chemotactic factors, which coincided with observed nerve regeneration and functional recovery **(30)**.

Not only has ADSC migration been concurrent with regeneration but primary nerve repair in rats demonstrated better regeneration with fat grafting compared to primary nerve repair alone according to sciatic function index and pinprick results. The injected fat was centrifuged autologous fat that received no chemical manipulation, which demonstrates the raw regenerative potential of adipose tissue **(31)**.

A slightly different introduction of ADSCs showed similarly promising results; when ADSCs were suspended in fibrin glue, the addition to epineural suture repair after transection in rat sciatic nerves of the enhanced glue was significantly superior in terms of myelinization, axon count, and muscle weight quotient than that of normal fibrin glue. Using fibrin glue has the additional benefit of providing extracellular support to the healing nerve **(32)**.

Another study evaluated the functional recovery of rat sciatic nerves after repair with and without ADSCs. A swim test revealed accelerated functional recovery in 2 weeks, with continued improvement at 6 weeks, for ADSC-treated repairs compared to non-treated repairs. Upon histological analysis, the ADSC injected group had significantly higher mean values for nerve fiber density, axon area, and myelin area **(28)**.

Nerve Grafting

The PNS possesses regenerative ability due to the presence of Schwann cells versus oligodendrocytes in the CNS and the continuous basement membrane on the outer surface of Schwann cells, termed the neurilemma **(33)**. However, in some more severe cases of injury, the PNS is unable to regenerate axons. The longer a nerve must travel to be fully regenerated, the slower the rate of regeneration. This phenomenon has to do with the distance from the cell body, which is where the cell machinery working to make new proteins is located, and then the cell will have to transfer these materials down its axons. There is a specific gap length, the critical-sized nerve gap, for each species in which the space is too large for a peripheral to be able to regenerate or reinnervate targets. Nerve gaps under the threshold number can successfully be repaired using a nerve graft **(9)**.

The rat critical nerve gap length is 1.5 cm, and the human is 4 cm. A human PN will naturally regenerate 1 mm/day, and animal nerves are assumed to regenerate at this rate as well. Autologous nerve grafts are the most reliable method of reconstructing nerve gaps, especially for sensory nerve gaps greater than 3 cm or for any motor nerve **(1)**.

Acellular grafts alone have not been as effective as autografts for longer grafts and the gold standard for motor nerves is to use a sensory nerve as a graft. The clinical repair rate neared 80% for nerve injuries repaired with autologous nerve grafts over a decade ago **(34)**.

Not all nerves repaired with grafts should be expected to have the same recovery rate. With intermediate-level repair in relation to the proximal stump, motor recovery potential was significantly greater for musculocutaneous (100%), radial (98.3%), and femoral (87.5%) nerves compared to tibial (63.9%), median

(52%), and ulnar (43.6%) nerves. In this way, the authors speculated that the characteristics of the effector muscle, nerve microanatomy, and topography of motor neurons within the spinal cord are the differentiating factors **(35)**.

In a study where nerve grafts taken from the other leg of the same rat were used to repair sciatic nerve injuries, an ADSC transplant demonstrated improved regeneration. Specifically, the ADSCs increased the survival of spinal L5 ganglia neurons by 26.4%, improved sciatic nerve vascularization by 35.68%, and increased the number of myelin fibers in the distal nerve by 41.87% **(36)**.

In a separate study, after transection and autologous graft repair in rats, an experimental group that received fibrin glue containing ADSCs after sutures had an 18% higher amount of myelin fibers in the distal nerve segment than the group that received fibrin glue without ADSCs 30 days after the surgery **(37)**.

Finally, in an experiment involving facial nerve lesions in rats, the sciatic nerve was used as a graft to connect the left and right marginal mandibular branches of both facial nerves. A group that was wrapped in an ADSC sheet had significantly higher amplitude when evaluated with evoked compound electromyography compared to both the group that received an ADSC suspension after the graft and the group that served as the control, with only a graft and no ADSC supplementation. The ADSC sheet group also had a significantly reduced time needed for reinnervation compared to the other two groups **(38)**.

Nerve Transfer

Nerve transfers should be considered for proximal nerve injuries and in injuries where there is concern that degradation of neuromuscular junctions will occur before reinnervation is possible. Transfers are classically used in brachial plexus injuries. Proximal injury to a nerve necessitates regenerating axons from the proximal stump to travel farther, which makes reinnervation of the distal target more difficult. In fact, in a paper that assessed the variability of nerve graft success, the proximal grafts were highly unsuccessful compared to intermediate and distal grafts **(35)**.

Performing a nerve transfer provides regenerating axons to the distal end of the injured nerve, bypassing the problem as well as overcoming graft length limitations **(39)**.

ADSCs have been experimented with in animal studies involving nerve transfers. In a study of rats, a cross-facial nerve graft, which is classified as a transfer, was performed by coapting a transected facial nerve to both ipsilateral and contralateral buccal branches, using the sural nerve as an interposition graft to bridge the distance **(40)**.

In one group, ADSCs were injected after the transfer was performed, and another group did not receive ADSC injection. The ADSC-injected group showed enhanced axonal regeneration evidenced by a statistically significantly increased improvement in whisking behavior compared to the control group **(40)**.

In an animal experiment in rats involving TENGs, brachial plexus injuries were repaired using a contralateral C7 nerve transfer combined with either unenhanced acellular nerve allografts or with acellular nerve allografts seeded with differentiated ADSCs. The group that received differentiated ADSCs showed improved compound muscle action potential and motor conduction velocity as well as histologically more neurofilament, S100, larger diameter axons, thickened myelin sheaths, and higher-density myelination **(41)**.

Another study transferred the phrenic nerve to restore the musculocutaneous nerve in C5-C6 avulsion injury in rats but instead used bone marrow stem cells in the treatment group to seed acellular nerve grafts. Interestingly, there were no differences reported between groups with acellular nerve grafts and groups where acellular nerve grafts were seeded with bone marrow stem cells (BMSCs) **(42)**.

Previously, both BMSCs and ADSCs have demonstrated the ability to differentiate into Schwann-like cells, so it is unclear why ADSCs showed histological and functional advantages in nerve transfer experiments when BMSCs did not. However, even in the case that BMSCs and ADSCs were to demonstrate similar outcomes in experiments, ADSCs have the benefit of being more easily accessible **(43)**.

Nanofat in Plastic Surgery

Nanofat is an emerging technique for fat grafting that is gaining popularity in the fields of regenerative medicine, aesthetics, and translational research. This treatment became known as fat grafting, lipofilling, and lipomodelling **(44)**.

The power of mesenchymal stromal/stem cells (MSC) has been known since the 1970s and recent studies have shown that MSCs are present in multiple tissues such as adipose tissue. Indeed, adipose tissue is a major reservoir of MSCs and is accessible via liposuction techniques daily used in plastic surgery **(45)**.

Rigotti et al. in 2007 showed for the first time how the injection of adipose tissue in severe radiation lesions improved tissue hydration and neo-angiogenesis **(46)**. In fact, by producing appropriate support MSCs promoted neoangiogenesis and reduced tissue inflammation. Capitalizing on the properties of MSCs, Magalon et al. used this treatment to cure scleroderma **(47)**. On a parallel track, Yoshimura et al. used fat enriched with stromal vascular fraction (SVF) for breast augmentation with good results **(48)**.

The current clinical applications of the nanofat technique are multiple:

aesthetic, burns, arthritis lesions, and diabetic ulcers **(49)**.

Nanofat in Nerve Regeneration

Hence it has become increasingly evident that MSCs used by adipose tissue transfer have a considerable interest in regenerative surgery. A new era began when Tonnard et al. in 2013 described the mechanical digestion of adipose tissue, a simple process termed ―nanofat grafting‖. This technique is based on the use of autologous fat and represents a new concept in the field of lipofilling. In this technique, fat is harvested from the patient and transformed into nanofat, which is composed of small fat particles (less than 0.1 mm in diameter) containing a high concentration of stem cells and growth factors **(50)**.

Figure 1: This picture shows how adipose tissue is mechanically emulsified to obtain nanofat by the authors' method **(44).**

Subsequently, a number of studies used nanofat in nerve regeneration in animal models, showing its efficacy **(51)** however, more studies are needed to validate the use of this new technique.

The animal model has the advantage of having multimodal approaches and different combinations of methods to study axonal regeneration. In addition, the assessment of nerve regeneration using animals provides information for translational research and future therapeutic options available for humans. In PNI, the use of animals as models helps to achieve more results concerning different treatments because clinical data from humans can have economic, practical, or ethical limitations. For that reason, the correct use of animals has proven valuable for later human clinical trials in PNI, which happen both in humans and animals. **(52)**. For that purpose, we aim to assess the effect of autologous nanofat grafting on nerve regeneration in male albino rats.

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