REVIEW ARTICLE

Adipose derived stem cells for the peripheral nerve regeneration: review of techniques and clinical implications

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Abstract

Adipose tissue is considered as a multipotent organ with multiple cellular varieties, like adipose derived stem cells with ability to differentiate into nerve cells. This review is an attempt to summarize the techniques of harvesting, isolating and delivery of adipose derived stem cells to injured nerve area and various interactions involved in the release of neurotrophic and angiogenic factors from stem cells. Neuro-regenerative potential of ADSCs is explained on the basis of "Paracrine hypothesis", according to which ADSCs secrete multiple neurotrophic factors and upregulates secretion of these neurotrophic factors by Schwann cells, leading to improved myelination, regeneration and decreases nerve fibrosis. ADSCs are easily available in abundance and undergo multi-step processing before grafting to nerve injury site. Acute inflammation, hypoxia and co-culturing with Schwann cells promotes neural differentiation of ADSCs. ADSCs and Schwann cells are reported to have similar mitogenic and differentiation factors, moreover, the micro-environment containing various growth factors and extracellular matrix plays a crucial role in promoting myelin formation by stem cells.

Various animal model studies have shown improved outcomes when ADSCs were used for the management of peripheral nerve injuries after direct repair, nerve grafting, nerve conduit, nerve allograft. This review contains various pre-clinical studies that have shown outcomes of adipose derived stem cells in nerve regeneration in different grades of nerve injuries.

Keywords: Angiogenesis, Mitogens, Nerve Injuries, Inflammation, Allografts, stem cells.

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Introduction

Medical advancements in the field of regenerative medicine have proved the application of adipose based stem cells in numerous pathologies, with long term results. Adipose tissue is considered as a multipotent organ comprising of multiple cellular varieties with regenerative abilities like adipose-derived stem cells (ADSCs), adipose-derived stromal cells etc. ADSCs have the potential to differentiate into osteoblasts, neural cells, endothelial and epithelium. Easy availability, abundance, immunomodulatory and anti-inflammatory effects; autocrine and paracrine functions, the ability to differentiate into cell types that are specific to damaged tissue and organs, are few of the revolutionary properties of the stem cells derived from adipose tissue.[1]

Peripheral nerve injuries secondary to trauma or tumour surgery can cause significant morbidity to the patient and its management is a therapeutic challenge as even after surgical repair, results are sub-optimal. Constant research is being done to enhance the nerve recovery and regeneration of the injured peripheral nerve after surgery.[2] Regenerative ability of adipose stem cells in nerve regeneration has been confirmed by multiple studies as it aids in tissue repair and helps to restore the functional deficit. [3]

Lipofilling and plasma enriched with platelets have been reported for managing the neuropathic pain in peripheral nerve surgery and it is believed to improve tissue healing by increasing neovascularization.[4] Adipose tissue has multipotent progenitor cells and secrete growth factors that forms the basis of the bioengineering and cell-based therapy for peripheral nerve repair and healing.[5]

Various preclinical studies on animal models have proven the potential benefit of adipose derived stem cells in nerve regeneration secondary to the effect of neuropathic and angiogenic factors released by these cells. These factors decrease nerve damage while facilitating nerve regeneration post-operatively and create a favourable microenvironment for axon outgrowth and neurite regeneration.[6]

This review summarizes the current research findings on the techniques and applications of adipose derived stem cells for peripheral nerve regeneration. Moreover, it aims to present an overview of the scientific evidence and the outcomes of the use of adipose derived stem cells in peripheral nerve regeneration when trailed in animal

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models as well as clinical studies.

Search strategy and inclusion criteria

Literature review was done using PubMed, MEDLINE and Google Scholar databases. Following search terms were used: adipose derived stem cells, adipose stem cells, neural regeneration, nerve regeneration, nerve repair, peripheral nerve regeneration, neural stem cells. Most relevant English studies about adipose derived stems cells and peripheral nerve regeneration and repair were included until November 2022.

Paracrine hypothesis of adipose-derived stem cells

Survival of the grafted ADSCs or any stem cell is explained by the "paracrine hypothesis". According to this hypothesis, the paracrine factors secreted by an individual stem cell (stem cell secretum), are released in hypoxic, acute ischaemic and inflammatory conditions. These paracrine factors promote intercell communication and angiogenesis for graft survival. In contrast to the beneficial effect of acute inflammation for regeneration, chronic inflammation causes failure of graft uptake and fibrosis of tissue.[7-12]

Classification of nerve injuries

There are two main classifications of nerve injuries: Seddon[13] classification and Sunderland classification.[14]

Sunderland's[14] system classifies nerve injuries to five categories. A first-degree injury includes nerve injuries with segmental demyelination and is comparable to Seddon's neurapraxia. In second degree axons are severed but endoneurium intact and such nerve injuries have chances of regeneration, while in third-degree there is disruption of the axon and endoneurium with intact perineurium and fascicular. In fourth-degree injury there is loss of axon, endoneurium and perineurium, only epineurium is intact. There is a loss of continuity of nerve trunk in fifth-degree comparable to neurotmesis. Sunderland grading is used by surgeons to decide about non- operative vs operative intervention. Spontaneous recovery may occur in Sunderland's first- and second-degree injuries but unlikely in third degree injuries and impossible in fourth- and fifth-degree injuries, hence surgical repair is indicated. We will review the role of ADSCs in different degrees of nerve injuries.

ADSCs and neurotrophic factors

Nerve growth factor (NGF) was the first neurotrophic factor to be discovered from nerve cells. Neurotrophic factors like brain derived neurotrophic factor, ciliary neurotrophic factor and glial derived neurotrophic factor (GDNF) are normally released from the Schwann cells and are transferred in retrograde manner from axons to cell body. In case of nerve injury this movement halts and the neurotrophic factors supply stimulus for regeneration by guiding the advancing axons at the injured ends. Exogenous NGF is found to be associated with increased axonal regeneration and decrease neuronal death, whereas GDNF is linked with improved nerve conduction velocity of sensory and motor neurons.[15]

Culturing of Schwann cells with ADSCs upregulates the expression of NGF, BDNF and glial cell derived neurotropic factor (GDNF), also known as Schwann cell differentiating factors.

Presence of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), causes neurogenesis, by activating AMP-activated kinase pathway.[2,16] Another in vitro rat study, has confirmed that NGF present in ADSCs conditioned medium extracted from rats, is responsible for the neurite outgrowth in neurogenic cell line. [16]

In a rat sciatic nerve injury model, 3 groups of 5 rats in each group were managed with polydimethylsiloxane bridging conduits and each group was supplemented with ADSCs, laminin and ADSCs + laminin, respectively. Few weeks later, distal stump analysis showed thinner nerve fibres in only ADSCs group and higher density of nerve fibres in group supplemented with both ADSCs and laminin.[12]

Extra cellular matrix substances like fibronectin and laminin enhances neurite growth in primary sensory neurons in a rat model. ADSCs can itself secrete laminin and cytokine CXCL5, which when delivered in area with nerve injury, can promote myelination by Schwann cells and stimulate signalling among Schwann cells respectively. Ischaemia and oxidative stress stimulate bone morphogenetic protein-2 and fibroblast growth factor-2, that acts via p38MAPK pathway in human ADSCs to improve neurogenesis. Other growth factors can stimulate ADSCs to produce vascular endothelial growth factor and angiopoietin 1, required for angiogenesis and for axonal regeneration.[8,9,10,16,17.]

Schwann cell like differentiation of ADSCs

Schwann cells myelinate neurons in peripheral nerve cells and functions to regenerate neurons after injury. First report of similarity between ADSCs and Schwann cells was published by Kingham et al. In this study, enzymatic digestion of rat visceral fat was done to yield mesenchymal stem cells and when treated with glial growth factors, these stem cells developed morphology similar to Schwann cells.[18] Also, these cells expressed glial

cell markers S100B, glial fibrillary acidic protein (GFAP) and p75 neurotrophic receptors when treating with Schwann cell mitogenic and differentiating factors.[18-20] Co-culturing of ADSCs with degenerating sciatic nerves can induce Schwann cell like differentiation.[21] Mice studies have further shown that ADSCs can express specific peripheral myelin proteins when converted into the Schwann cell phenotype.[22] Along with the ability of ADSCs to differentiate into Schwann cells, the other significant effect of ADSCs on the peripheral nerve regeneration is due to release of various growth factors. [20]

Sources and harvesting of ADSCs

The first adipose tissue grafting was done to fill a scar depression by Neuber in 1893[23], since then adipose tissue grafting has been used for multiple clinical conditions. Adipose tissues are isolated from subcutaneous fatty tissues, which can be harvested by vacuum/syringe suction or surgical excision. Most common donor sites are lower abdomen, thighs and arm. Harvesting technique is based on the volume required as small volumes of adipose tissue, can be harvested using syringe suction, whereas large volumes of lip aspiration is done by liposuction devices. Preserving the viability of harvested adipocytes is common goal in all techniques as poorly harvested cells are damaged with decreased adipogenic differentiation ability.[24]

Adipose tissue grafts are non-vascularized and therefore few adipocytes at centre of the graft undergoes fat degeneration and necrosis within the 24 hours, due to ischaemia. Like every other graft, initial stage of adipose tissue graft 'take' is imbibition, however there is no stage of inosculation and vascular ingrowth is induced from surrounding existing vascular network.[25] Adequate plasma diffusion from the surrounding tissues is the key factor in the survival of grafted adipose tissue, hence greater volume adipose tissue graft has a higher chance of necrosis and macrophages mediated resorption due to decrease plasmatic diffusion to adipocytes. Therefore, small aliquots fat grafting in multiple session is preferable over large volume grafting in single session.[26] Adipose graft survival is also dependent on the patient's age, co-morbidities, harvesting techniques, body mass index etc. One gram of adipose tissue has on average 5000 ADSCs, and ADSCs derived from adipose tissues of different body regions have unique characteristics.[27]

Separation and delivery of ADSCS from adipose tissue

Adipose tissue either lipoaspirate or surgically excised, undergoes series of steps to extract ADSCs. These steps include sedimentation, filtration, centrifugation (force= 1200 x g for at least 3 minutes), followed by enzymatic digestion with collagenase. Centrifugation yields three layers; first layer has debris and lipids of damaged adipocytes, second layer has adipose tissue and third layer i.e the layer of interest contains blood, tumescent and stromal vascular fraction (SVF) pellet. Further processing is needed for the digestion of non-fat components like blood and collagen fibres as, they can cause inflammation at the recipient site. SVF derived from adipose tissue consists of mature fat cells, ADSCs, immune cells, epithelial cells and endothelial cells.[2,28] SVF contains < 0.1% ADSCs and other stem cells with potential to differentiate to other cell lineages.[29] Flow cytometry is used to identify different cell populations in extracted SVF. Multiple surface markers are used to decide the subpopulation of ADSCs, endothelial progenitor cells, pericytes as none of them has any unique surface marker. DAPI marker helps to exclude apoptic cells from further analysis. The positive markers of ADSCs are CD90, CD44, CD29, CD105, CD13, CD73, and the negative markers are CD31, CD45, CD14, CD11b, CD34, CD19, CD56, and CD146. The positive markers of the pericytes are $CD146^{+}$, $CD90^{+}$ and negative markers are $CD31^{-}$, $CD34^{-}$.[30]

ADSCs derived from perinephric fat, when compared to subcutaneous fat from elsewhere in the body, is found to be linked with upregulation of all three glial cell markers like Schwann cells.[31] However, another study has reported that the ADSCs isolated from the superficial layer of abdomen are superior to those taken from the deep layer, in their neurite outgrowth-enhancing properties, but no specific neurotrophic factor was found to be responsible for this characteristic.[32]

Once ADSCs have been derived the method of its delivery to the target tissue is controversial. It can be delivered via intravenous injection,[21] direct intra-neural injection or dynamic seeding. Intra neural injection delivers high quantity of ADSCs to the nerve, with variable distribution. Also, most of the ADSCs are believed to be damaged after passing through micro injection, therefore slow injection is advisable. Another method of the delivery of stem cells is dynamic seeding, in which stem cells and nerve segments are placed on a rotator, in a tube, for 12-24 hours. This leads to the adherence of the stem cells to the outer surface of the nerve. Efficacy of stem cells when delivered in the form of gel is a non-invasive technique, however it is still under research.[33]

Role of ADSCS in the management of peripheral nerve injuries

Management of Sunderland's Grade 1 and Grade 2 injuries

Adipose tissue grafting and adipofascial flaps are classically used for recurrent or persistent carpal tunnel pain. There are multiple reasons for failed carpal tunnel release like incomplete release, extensive scarring, fibrosis of skin etc. Many studies have reported successful results of extensive neurolysis and lipofilling adjacent to the nerve, in recurrent carpal tunnel syndrome. Advantages of this technique are decrement in collagen production, minimally invasive, and ADSCs in grafted adipose tissue stimulates nerve regeneration directly.[34,35]

Surgical management of Sunderland's Grade 3-5 injuries

Surgical repair of peripheral nerve should be directed to decrease the tension in the suture line as it will lead to increased fibrotic reaction and poor regeneration.[36]

• Direct repair of nerves

Direct nerve repair is recommended, in cases of nerve transaction, when two nerve ends can be held without tension. Regenerative medicine has been exploring to improve outcomes for the patients after primary nerve repair. In a rat study model, it was observed that administration of systemic ADSCs after peripheral nerve primary repair, results in improved motor function, nerve regeneration and nerve repair when compared to the group in which nerve repair was not supplemented with ADSCs.[37]

• Repair by nerve grafting

This is currently the gold standard treatment for any peripheral nerve injury with the loss of trunk. If nerve loss in an injury is 2 cm and primary repair cannot be done due to high tension, then use of nerve graft is indicated. Common types of nerve grafts are cable grafts, trunk grafts and vascularized nerve grafts. Vascularized grafts are better indicated for nerve gap of more than 6 cm. The nerve graft should be 10-20 % more in length than nerve gap to adjust for shortening secondary to fibrosis.[15] In a rat study model, it was demonstrated that in the case of sciatic nerve injury when nerve grafting is done and ADSCs are delivered to this area of injured nerve by using fibrin glue, leads to increased neuron survival, regeneration and myelination.[38] Another study has reported similar findings on assessment of wrapping of ADSCs loaded hydrogel around 20 mm autograft.[39]

• Repair by nerve conduits

This is a relatively a newer and emerging concept for the management of nerve injuries. Nerve conduits are used for the nerve gap of 3-4 cm. These conduits can be biological (vessels, laminin, collagen, fibrin etc.) or synthetic (polyester etc.), which are further classified as first, second and third generation conduits.[15,40] Neurotropism allows proximal end of regenerating nerve to selectively grow towards desired distal stump. Nerve conduits provide comparable results to the nerve grafting in small peripheral nerves with small diameter. This is associated with decrease donor site morbidity present in nerve grafting.[41]

A trial was performed on rats in which 10 mm sciatic nerve gaps were made and then repaired with FDA approved type 1 collagen conduits. In first group of 7 rats, conduits used were pre-seeded with ADSCs and in second group, acellular conduits were used. Motor and sensory nerve conduction velocity was assessed after 6 months that showed superior outcomes in nerve regeneration and nerve conduction velocity in nerve gaps managed with ADSCs pre-seeded conduits.[10]

Fibrin conduits made up of fibrin glue when provided with ADSCs to reconstruct 10 mm defect of common peroneal nerve in rats, demonstrated low levels of collagen deposition at the distal side of conduit on histological level, when compared to the non-ADSCs nerve conduit group.[42] Not only this, the extracellular matrix in ADSCs treated conduit become more organized around the regenerating neural tissue.[10,42] Similar to ADSCs, SVF, also improves anatomical and functional outcomes in neural regeneration than empty nerve conduits. In an original study, when the nerve conduits for facial nerve injury in rats were treated with SVF, showed highest number of myelinated fibres.[43] Degree of peripheral nerve axonal regeneration can be evaluated by immunohistochemical staining agents like growth-associated protein-43, neurofilament, S-100, GFAP, β-III tubulin, and laminin.[44]

• Repair by nerve allografts

Bain et. al has reported the use of nerve allografts for peripheral nerve injuries in primates.[45] Saffari TM et.al reviewed the patterns of revascularization of allograft nerves in 51 rats with sciatic nerve injury and it was found that the group of rats in which wrapping of adipofascial flap was done around nerve allograft resulted in better survival of nerve allograft by increasing revascularization when compared to two other groups in which sciatic nerve injury was managed with autograft and allografts without flap. This is due to the effect of various growth factors released from subcutaneous adipose tissue, causing decreased fibrosis and increasing myelination. [46] In a dog study model of sciatic nerve injury, nerve allografts when treated with ADSCs and Schwann cell-derived neurotrophic factor showed significant S100 and

GFAP expressions, indicating proliferation of Schwann cells.[47]

Adjunctive therapies with ADSCs

• Role of immunomodulators in nerve regeneration

Local and systemic use of the immunosuppressive drug FK 506 (tacrolimus) has been shown to accelerate nerve regeneration and functional recovery. It acts through FK 506-binding protein (FKBP) receptors. Local use of FK 506 enhances nerve regeneration after nerve repair in rat models.[48,49]

Yan et al. in his experimental study on rats, showed a significant therapeutic effect in the short-term use of FK 506 in nerve regeneration. In the future, the use of FK 506 may serve as an important role as an adjunct to nerve allografts.[50]

• Role of Platelet-Rich Plasma in nerve regeneration

PRP is derived from centrifugation of patient's blood that separates platelets, suspended in a small volume of plasma. This concentrate contains various growth factors: epidermal growth factor, insulin-like growth factor-1, platelet derived growth factor, transforming growth factor-β 1 and 2, thrombospondin and vascular endothelial growth factor.[51] Synergistic use of PRP with ADSCs for tissue regeneration improves ADSCs function by promoting vasculogenicity, under the influence of VEGF.[52] Also, PRP was found to enhance nerve regeneration property when given in combination with ADSCs in rat sciatic injury model. Axonal regeneration and myelination in this group was superior to untreated control group (not treated with either PRP, or ADSCs or nothing).[53]

Limitations for the use of ADSCs

Many in vitro studies have linked adipocytes and ADSCs with local tumour recurrence and metastasis, when used for reconstruction after breast cancer surgery. ADSCs when interact with cancer cells stimulate secretion of interlukin-6 from stem cells, which act in a paracrine fashion on tumour cells and enhance their malignant properties.[52,55] However, in clinical practice, autologous fat grafting with flap reconstruction, is declared safe enough to be used after oncological mastectomy, without any increased risk of locoregional recurrence. Considering the possibility of promoting tumour growth, still a lot of research is required to understand the long-term effects of ADSCs use in malignant conditions in humans.[56,57] Most of the studies involving the role of adipose derived stem cells in nerve regeneration are conducted on animals and is therefore a poor predictor of human reaction to the exposure of ADSCs in acute nerve injuries.

Future of ADSCS for peripheral nerve regeneration

Despite advancement in the techniques of surgeries, the lack of clinically available effective therapies following any nerve surgery, remains a clinical challenge. Often options for severe nerve injuries are limited and frequently do not result in a satisfactory functional recovery. Adipose tissue derived stem cells therapies have demonstrated the supportive role in nerve regeneration with a considerable window for clinical applications. The scientific evidence from in vivo and in vitro studies proves that the adipose tissue derived stem cells have the best potential to be used in nerve regeneration, however, translating these research findings into clinical use, is still under process.

The past decade had been about the scientific validity of the use of stem cells in different scenarios and in future, more human based studies are required for the advancement in integration of adipose tissues derived stem cells in surgical practice for nerve regeneration, with currently explored combination and synergistic effect of the therapies.

Conclusion

This review shows that various original preclinical studies have confirmed that ADSCs have significant regenerative potential in animals with acute peripheral nerve injuries by improving myelination, regeneration and decreasing nerve fibrosis. Acute inflammation, hypoxia and co-culturing with Schwann cells neural differentiation increases regenerative properties of ADSCs. Future therapeutic and clinical use of adipose derived stem cells can be justified based on their direct release of neurotrophic factors and indirectly by making changes in the micro-environment and upregulation of Schwann cells.

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