

PERIPHERAL NERVE REGENERATION: CELL THERAPY AND NEUROTROPHIC FACTORS

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ABSTRACT

Peripheral nerve trauma results in functional loss in the innervated organ, and recovery without surgical intervention is rare. Many surgical techniques can be used for nerve repair. Among these, the tubulization technique can be highlighted: this allows regenerative factors to be introduced into the chamber. Cell therapy and tissue engineering have arisen as an alternative for stimulating and aiding peripheral nerve regeneration. Therefore, the aim of this review was to provide a survey and analysis on the results from experimental and clinical studies that used cell therapy and tissue engineering as tools for optimizing the regeneration process. The articles used came from the LILACS, Medline and SciELO scientific databases. Articles on the use of stem cells, Schwann cells, growth factors, collagen, laminin and platelet-rich plasma for peripheral nerve repair were summarized over the course

of the review. Based on these studies, it could be concluded that the use of stem cells derived from different sources presents promising results relating to nerve regeneration, because these cells have a capacity for neuronal differentiation, thus demonstrating effective functional results. The use of tubes containing bioactive elements with controlled release also optimizes the nerve repair, thus promoting greater myelination and axonal growth of peripheral nerves. Another promising treatment is the use of platelet-rich plasma, which not only releases growth factors that are important in nerve repair, but also serves as a carrier for exogenous factors, thereby stimulating the proliferation of specific cells for peripheral nerve repair.

Keywords - Peripheral Nerve System/injuries; Regenerative Medicine; Nerve Regeneration

INTRODUCTION

Peripheral nerve transection traumas are extremely common in clinical practice and recovery without surgical intervention is rare. Lesions with loss of nerve substance produce serious problems for the patient. Besides causing pain and morbidity, these injuries usually generate permanent sequelae, such as sensory deficit and functional dysfunction. These lesions cause damages that substantially diminish the quality of life of these patients, including physical disability and total or partial loss of their productive activities, which gives rise to important social and economic consequences⁽¹⁾. The current repair techniques offer random and frequently unsatisfactory results. In view

of these limitations, many researchers seek therapeutic options to improve the repair of lesions with peripheral nerve transections⁽²⁾.

Nowadays autologous peripheral nerve transplantation represents the gold standard of repair when there is loss of substance that precludes neurotaphy. However, it presents some limitations, such as the need to perform two surgical procedures at different sites, the consequent greater morbidity and the shortage of nerve donor sites, besides the resulting sensory deficit in the area from which it was removed^(2,3).

In cases where the extent of the lesion precludes the simple joining of the stumps, an available and widely used repair technique is tubulization. This technique,

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Study received for publication: 2/11/2011, accepted for publication: 6/16/2011.

The authors declare that there was no conflict of interest in conducting this work

This article is available online in Portuguese and English at the websites: www.rbo.org.br and www.scielo.br/rbort

also called entubulation, is a surgical procedure in which the sectioned nerve stumps are introduced and fastened inside a tubular prosthesis, aiming to provide a favorable environment for regeneration. It also serves as a guide for the nerve growth of the broken or sectioned ends^(2,4), protecting the nerve fibers of the scar tissue and avoiding neuroma formation⁽⁵⁾. Tubulization presents another interesting characteristic: it can be optimized with the addition of regenerative factors⁽⁶⁻⁸⁾.

It is known that tissue repair requires a complex interaction between cells, extracellular matrix and trophic factors, which are all important elements involved in nerve regeneration⁽⁹⁾. Consequently, cell therapy and tissue engineering have been receiving a great deal of attention in recent decades, and are widely used in different areas^(7,10-13).

Although the complexity of molecular and cellular events of tissue repair is not yet completely clarified, existing knowledge of the mechanisms of the cascade that induces regeneration after peripheral nerve lesions is vast, and provides important information for a better conception of nerve repair. Therefore, the aim of this review is to provide a survey and analysis of experimental and clinical studies regarding the results obtained from peripheral nerve repair techniques, which use cell therapy and tissue engineering as tools to optimize the regeneration process. The articles used are from scientific databases LILACS, Medline and SciELO.

REVIEW OF LITERATURE

Cell therapy and peripheral nerve repair

Cell transplantation is one of the cell therapy and tissue engineering strategies aimed at the creation of a favorable microenvironment for tissue regeneration. Stem cells have important characteristics that differentiate them from other cell types, are undifferentiated precursor cells that have self-renewal ability and can differentiate into multiple lineages⁽¹⁴⁾. They are present in several tissues and are responsible for their regeneration in the event of injuries or lesions⁽¹⁵⁾. Bone marrow, adipose tissue, umbilical cord blood and peripheral blood are some sources of stem cells; however, these cells can be tissue-specific, i.e., originating directly from specialized tissues^(6,14,16-18). In nerve repair, the most widely used

cells include the mesenchymal cells of the bone marrow and of the adipose tissue, as well as the actual Schwann cells^(6,7,19-33) (Table 1).

These cells can be applied directly after density gradient separation (Ficoll-Paque[®]) or be cultivated and differentiated in vitro for subsequent application, as is the case of stem cell differentiation into Schwann cells. We present below a description of some of the cell types used most often in nerve repair surveys.

Table 1 – Types of cells used as cell therapy in nerve repair.

Cell	Study model	Reference
Bonemarrow-derived mesenchymal	Clinical	7
Bonemarrow-derived mesenchymal	Experimental	6, 19, 20, 21, 22, 23, 24
Adipose-derived mesenchymal	Experimental	25, 26
Schwann cells	Experimental	27, 28, 29, 30, 31, 32, 33

Schwann cells in nerve repair

The cells most commonly used in nerve regeneration are autologous Schwann cells (SC). These represent glial cells in the peripheral nervous system, and their main function is to provide support to the axons through the release of growth factors and isolation of the axon through formation of the myelin sheath⁽³⁴⁾. The addition of SCs in synthetic tubes assists regeneration in nerve defects, although repair with autologous graft, in most studies, still presents superior recovery^(27,29,33,35-37). Besides synthesizing growth factors, SCs also are able to produce extracellular molecules, such as laminin and type IV collagen. The extracellular matrix can serve as a reservoir of growth factors that are secreted by SCs⁽³⁸⁾.

Experimental studies based on the use of SCs as a therapeutic option for the recovery of nerves with loss of substance proved the efficacy of these cells^(28,32,39). SCs play an important role in the maintenance, nutrition and in the repair of peripheral nerves. Although there are still limiting factors in the use of SCs, these have promising results in tissue, physiological and functional improvement in

lesions caused by trauma or pathologies in peripheral nerves.

Bone marrow mesenchymal cells and nerve regeneration

Many researchers have developed studies on stem cells^(19,25,27,40). Embryonic stem cells, as well as those obtained from adult cerebral tissue, are able to undergo expansion and neuronal differentiation in vitro and in vivo. However, the inaccessibility of these cells limits their clinical use, which stimulated the search for cells that are capable of differentiating into neuronal lineages^(28,41). Moreover, there is controversy involving research with the use of stem cells concerning the sources from which these are obtained. In particular, the use of embryonic stem cells, although legally regulated in Brazil, allowing research into and the manipulation of these cells obtained through “unviable embryos”, is still a subject of ethical and political discussions^(29,30,42,43).

An alternative and viable source of mesenchymal stem cells is bone marrow. Adult bone marrow-derived cells are characterized as multipotent, as they are able to differentiate into cell lineages of mesodermal origin^(44,45). Several surveys on transplantation of bone marrow-derived mesenchymal stem cells (BMDMSC) have reported that these cells also present in vitro neuronal differentiation ability, which means they can be used in peripheral nerve repair⁽⁴⁶⁻⁵²⁾. Additionally, Montzka et al⁽⁵³⁾ demonstrated the ability of human BMDMSCs to express different neuronal and glial cell markers.

Experimental studies in rodents^(20,21,23), rabbits^(22,54) and primates⁽⁵⁵⁾ prove the efficiency of these cells, presenting positive functional outcomes in peripheral nerve repair. The combination of bone marrow mesenchymal cells with bioabsorbable tube increases nerve regeneration and sciatic nerve functional recovery in mice⁽⁵⁶⁾. BMDMSCs can positively influence the regeneration of peripheral nerves not only through the direct release of neurotrophic factors, but also through indirect modulation of the behavior of SCs^(24,46).

There is clinical evidence indicating BMDMSCs as an effective treatment in peripheral nerve repair. In comparing the tubulization technique with and without the addition of bone marrow mononuclear cells (this group of cells contains a fraction of stem cells) in 44 patients with damage to the median or ulnar nerve, it was verified that lesions treated with these cells

presented better results in the regeneration process than conventional tubulization⁽⁷⁾.

However, BMDMSCs present some limitations. Besides the fact that these cells are obtained with the application of epidural or general anesthesia, since harvesting occurs through a very painful procedure, the quantity of cells acquired often fails to reach the necessary number⁽⁵⁷⁾.

Adipose tissue-derived mesenchymal cells and nerve regeneration

Mesenchymal stem cells are not only present in the bone marrow, but also in other tissues including the adipose tissue⁽¹⁸⁾. Most authors call them adipose-derived stem cells, or ADSCs.

There is a strong resemblance between ADSCs and BMDMSCs, since both present a similar immunophenotypic profile, as well as the ability to differentiate into osteogenic, chondrogenic, myogenous and adipogenic lineages^(58,59). The advantage of using ADSCs is their widespread availability, as human subcutaneous fat is abundant, and can be harvested easily through the liposuction procedure, besides the fact that it appears much more frequently in the adipose tissue than mesenchymal cells in the bone marrow⁽⁶⁰⁾.

The ability of mesenchymal cells originating from the adipose tissue to differentiate into cells with neuronal features was proved in vitro. Kingham et al⁽⁶¹⁾ reported that ADSCs are able to differentiate into cells similar to the genuine SC, when cultivated together with a combination of glial trophic factors.

Cells derived from adipose tissue of murinae and humans, after neuronal induction, presented morphology typical of nerve cells, which were positive for immunocytochemical expression of GFAP, nestin and neuron-specific nuclear protein (Neu-N). Pre-treatment with epidermal growth factor (EGF) and basic fibroblast growth factor (FGF) increases the neuronal differentiation of adipose-derived human stem cells, whose use has important biological and clinical implications⁽⁴¹⁾. However, the adipose-derived mesenchymal stem cell should have the ability to produce myelin sheath, the main function of SCs. This characteristic was confirmed in in vitro studies, thus becoming another option for the treatment of peripheral nerve injuries⁽⁵⁷⁾.

The adipose-derived mesenchymal stem cells present results similar to bone marrow-derived cells in vivo^(25,26). However, due to the ease of their harvesting

and abundant quantity of cells available in subcutaneous deposits, ADSCs are indicated as a better alternative for clinical application⁽²⁵⁾.

Growth factors in nerve repair

The complexity of nerve regeneration involves a range of elements that interact with one another, and are all essential to the process; among them, the growth factors (GF) aroused a great deal of interest in the scientific community⁽⁶²⁾, due to their performance as important cell modulators⁽⁶²⁻⁷⁸⁾ (Table 2).

Degenerated peripheral nerves are an important source of these factors, as are the SCs. These proteins are basically a set of three families of molecules and their receptors, responsible for maintaining the growth and survival of the sensory and motor axons and neurons after tissue damage⁽⁶²⁾.

The local presence of GFs is important in the control of the survival, migration, proliferation and differentiation of various types of cells that are engaged in nerve repair^(9,62). For these reasons, the use of therapies based on GFs has increased in the last few decades. Growth factors should be administered locally to achieve a more adequate therapeutic effect with few adverse reactions. Therefore, the delivery of growth factors for nerve regeneration can be ideally combined with nerve conduits⁽³⁴⁾.

Among neurotrophic factors, the nerve growth factor (NGF) is the most researched factor, due to its action in the proliferation and differentiation of neurons⁽⁶⁴⁾ and as it assists in the repair and functional recovery of injured nerves⁽⁶³⁾. When combined with biomaterials and with controlled release, its effect can

be strengthened⁽⁷⁹⁻⁸¹⁾. The ability of the NGF to promote functional recovery after lesions was confirmed in experimental studies^(65,81).

The endogenous brain-derived neurotrophic factor (BDNF) demonstrates an important role in the induction of the cell body response in injured rat neurons. When exposed to mitogens such as BDNF, stem cells differentiate into neuronal lineages *in vitro*⁽⁶⁸⁾.

The glial cell line-derived neurotrophic factor (GDNF) is considered the most protective factor for motor neurons⁽⁷⁰⁾, and is essential in their formation, as well as that of sensory neurons during the regeneration process⁽⁸²⁾. GDNF has its expression elevated in experimental models of motor neuropathies in rats, several human neuropathies and in traumatized human nerves⁽⁸²⁾. In a model of peripheral nerve lesion in rats, it was demonstrated that the combination of nerve conduits composed of chitosan, GDNF and laminin was significantly more efficient during the initial stages of nerve repair, promoting greater axonal growth and myelination in six weeks after the animals' nerve transection⁽⁸³⁾.

The ciliary neurotrophic factor (CNTF) assists in the differentiation and in the survival of a variety of neurons, and the mRNA expression levels of CNTF decrease significantly and continue low for a long time after peripheral nerve transection⁽⁷¹⁾.

Similarly, the insulin-like growth factor (IGF) also assists in nerve regeneration. IGF-1 is present in several stages of development of the peripheral nerve system, performing a wide range of functions, including the promotion of the regeneration of motor and sensory axons⁽⁷²⁻⁷⁵⁾. Evidence suggests that high levels of IGF in denervated muscle can stimulate regeneration with nerve sprouting⁽⁸⁴⁾.

Besides acting essentially in the vascular tissue, the vascular endothelial growth factor (VEGF) also assists nerve regeneration, due to the close relationship existing between the nerve fibers and the blood vessels during this process. The addition of VEGF significantly increases the infiltration of blood vessels in nerve conduction chambers, and is related to the increase of axonal regeneration and migration of SCs^(76,77). Moreover, the VEGF acts as a neuroprotective agent in neurons *in vitro* after ischemic lesion⁽⁷⁸⁾. In an experimental study,

Table 2 – Main neurotrophic factors used in peripheral nerve repair.

Growth factor	Main target	Reference
NGF	Sensory neurons and small axons	63, 64, 65
BDNF	Sensory neurons and large axons	66, 67, 68
NT-3	Sensory and motor neurons	69
NT-4/5	Motor neurons	62
GDNF	Motor neurons	70
CNTF	Sciatic nerve	71
IGF	Inflammatory (anti-inflammatory) cells; sensory and motor neurons	72, 73, 74, 75
VEGF	Vascular endothelial cells	76, 77, 78

the use of VEGF demonstrated an effect on vascular blood supply, with a significant increase of axonal regeneration and of SCs, stimulating nerve regeneration⁽⁸⁵⁾.

Collagen and laminin as carriers

Components of the extracellular matrix are collagen and laminin, essential for guidance and axonal growth during the nerve regeneration process. Collagen and laminin are involved in the regeneration process through the formation of a substrate for the migration of non-neuronal cells. The filling of silicone tubes with these components presents an increase in the regeneration rate⁽⁸⁶⁾ and in the connection of extensive defects⁽⁸⁷⁾. This effect, however, depends on some factors such as the concentration and permeability of the tube^(87,88).

Nowadays, different gels containing collagen or laminin (Matrigel[®]) are being used as a support for cells and growth factors^(27,35,37). Collagen, as the main component of the extracellular matrix, is used in various surgical prostheses. A study on an animal model demonstrated the efficacy of a biological matrix composed of collagen (Tissudura[™]) when used in nerve regeneration⁽⁸⁹⁾.

As is the case of collagen, laminin also plays an important role in *in vivo* axonal growth. Surveys include this component of the extracellular matrix in animal models to repair injured sciatic nerves and to obtain an improvement in some areas of axonal regeneration. The application of tubes composed of chitosan combined with laminin demonstrates that the tube optimizes the nerve regeneration process during the initial phases of repair⁽⁸³⁾.

Use of platelet-rich plasma in peripheral nerve repair

Autologous blood-derived platelet-rich plasma (PRP) is defined as a volume of plasma with platelet concentration around five times above the physiologic levels⁽⁹⁰⁾. The platelets that constitute PRP are able to release various growth factors that are essential for the healing of lesions, such as the three platelet growth factor isomers (PDGF $\alpha\alpha$, $\beta\beta$ and $\alpha\beta$), VEGF, transforming growth factor (TGF- β 1 β 2) and epithelial growth factor (EGF)^(91,92). The platelets are also responsible for the synthesis and storage of BDNF⁽⁹³⁾.

PRP has been used by areas such as oral and bu-

comaxillofacial surgery for some time⁽⁹⁴⁻⁹⁶⁾, and it has aroused considerable interest in cosmetic^(97,98) and orthopedic^(99,100) surgery. In experimental studies, PRP was used in peripheral nerve lesions, promoting remyelination in the facial⁽⁹¹⁾ and sciatic nerve of rats⁽⁸⁾.

The application of PRP increases the number of nerve fibers after peripheral nerve lesions, and can produce a neurotrophic effect, stimulating the proliferation of Schwann cells and myelination, important components during peripheral nerve repair^(6,101).

Data in literature on the effect of PRP on peripheral nerve regeneration are scarce, which stimulates the search for more precise information about its performance. Therefore, this treatment should receive attention and be expanded, as it has the potential to become another safe option of low associated cost for the treatment of a wide variety of lesions and neuropathies in peripheral nerves.

CONCLUSION

The concept of an ideal treatment to assist in nerve repair is based on the creation of synthetic tubes, preferentially bioabsorbable, covered by components of the extracellular matrix and that are appropriate for controlled release of one or more neurotrophic factors, bioactive elements or cells. The combination of two or more growth factors probably has a synergic effect on nerve regeneration, especially when they belong to different families and act by distinct mechanisms. In spite of the vast knowledge already acquired about these proteins in the improvement of nerve regeneration, more experimental studies are necessary before their use in clinical practice.

The use of cells, whether actual Schwann cells or the stem cells obtained from varied sources, demonstrates considerable benefits in the repair of peripheral nerves, with great potential to become one of the most promising options at the clinic.

Another interesting technique that has not yet been fully explored is the use of PRP, which releases autologous growth factors and serves as a carrier for other exogenous factors in nerve regeneration.

Considering the evidence found, it was observed that a promising treatment is based on the combination of biological and synthetic elements for regeneration to be optimized and to provide better results.

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