REVIEW

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Schwann cell cultures: recent advances and novel approaches to the reconstruction of peripheral nerve defects

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Abstract

Current techniques in tissue engineering may offer a choice regarding the reconstructive strategies of peripheral nerves. Schwann cell cultures are to be considered an appropriate option in the reconstruction of peripheral nerve and spinal cord large defects. Schwann cells availability from peripheral nerve autografts creates a great benefit concerning their usefulness in the nervous autogenic transplantation. Allogeneic Schwann cells transplantation can be effective in the reconstruction without delay of peripheral nerve defects.

Keywords: peripheral nerve defects, Schwann cell cultures, peripheral nerve reconstruction.

Introduction

Peripheral nerve traumas highly embarrass the society. Common treatment involves termino-terminal nervous suture for injuries resulting in minor defects. Autologous graft is necessary in large defects. Although surgery techniques have met tremendous progress lately, functional regeneration has frequently been in deficit. Therefore, tissue engineering techniques advances in the last 15 years may be considered as choices in peripheral nerve regeneration strategies [1, 2].

Schwann cells are involved in important visible features concerning peripheral nerve biology: conducting the nervous impulse along axons, nerve development and regeneration, trophic support for neurons, extra-cellular matrix production, modulating the activity of neuromuscular synapses.

The main spindle in peripheral nerve regeneration consists of Schwann cell [3, 4], which is a dramatic therapeutic target as well. In interrupting the normal interaction between the axon and Schwann cell, by means of aggression upon the nerve, Schwann cells differentiation and nerve proliferation factors are promoted.

Revitalizing the method; clinical and experimental findings

Theodor Schwann discovered in 1839 some very special and peculiar cells (called Schwann cells) providing myelination of peripheral axons. Schwann cells precursors were found in developing stem cells within neural crest. When connected to nervous fibers, Schwann cells or precursors led to myelination of peripheral axons (each cell myelinating a segment of the axon) [5, 6].

Schwann cells degeneration was to be noticed because of peripheral nerve trauma. However, as the peripheral axons regenerated, Schwann cell precursors appeared in response of the axon to inflammation. Some findings showed that even Schwann cells degeneration was responsible for macrophage attraction towards the degenerated nerve, therefore leading to proinflammatory cytokines. Consequently, Schwann cells proliferation, including tumoral proliferation, was stimulated.

Dick and Mary Bunge [5] pioneered the studies concerning Schwann cells, within Miami Project. They succeeded in performing the first Schwann cell culture and were responsible for many current methods of their isolation and transplantation. Many researchers speculated that the difference in nerve regeneration between central and peripheral nervous system was caused by the difference between Schwann cell and oligodendroglia.

Schwann cells availability from autografts of peripheral nerves created a great benefit concerning their usefulness in nerve autogenous transplantation. Therefore, peripheral nerves were sacrificed (within the limits given by the donor: sural nerve, etc.). But, the harvested peripheral nerve did not possess many Schwann cell precursors. Therefore, it could be taken into account that Schwann cell culture dramatically provided such precursors.

Allogeneic Schwann cells transplantation

Schwann cell cultures were considered a promising

field for the reconstruction of peripheral nerves or nerve regeneration in the central nervous system injuries. The initial cell cultures were made of embryonic or neonatal cells [7, 8].

The use of Schwann cells in surgical reconstruction of peripheral nerve involved the use of adult cells, in order to prevent the allograft allogeneicity. Unfortunately, these cells were difficult to isolate and cultivate. This difficulty started from the abundance of connective tissue and the highly differentiated state of the cells. Few from the isolated cells were suitable for cell culture. In their study, Ansselin AD *et al.* used 4–5-month-old rats, whose sciatic nerve was surgically injured, and cells were harvested from the distal stump of the conditioned nerve, after 10–12 days, when Wallerian degeneration was triggered. The cells could be cultured and isolated due to enzymatic separation [7, 8]. Although the cell culture supported the nerve regeneration, the results were uneven positive, with a relative low success rate [7–9]. Seemingly, the method supported peripheral regeneration, without immunosuppressant secondary effects, the results being inferior to allograft subjected to denaturation and temporary immunosuppression .

Furthermore, in 1998, Hadlock T *et al.* [10] presented their findings consequent to the reconstruction of peripheral nerves using tubes of poly-L-lactic acid in which Schwann cells (isolated, expanded in culture, and plated onto these polymer films) were implanted. The experiment showed nerve regeneration by polymeric complexes in Lewis rats, being carried out in order to establish its clinical availability in facial nerve reconstruction.

Cell cultures were obtained by harvesting sciatic nerves from sacrificed rats, by detaching the cells from epineural structures and fragmenting the remaining tissue in 1 mm segments, furthermore expanded in culture. In six weeks time, the explants were enzymatic digested, purified and expanded. This technology was applied in adult cells as well, with low results [10]. Therefore, as in the first experiment, the cultures relied on neonatal cells [7, 10].

Allogeneic Schwann cells transplantation could support the peripheral nerve defect reconstruction without delay. A method for enhancing the survival and/or proliferation of human Schwann cells in cell cultures was documented in Mather JP *et al.* (2000) [11]. The cells were cultured in a saline medium comprising gas6 and other mitogenic agents (heregulin and forskolin). The culturing step was preceded by a pre-incubation period (wherein demyelination occurred). Therefore, isolated Schwann cells could be used as cellular prostheses to treat patients with nervous system injuries. The authors detailed the culture medium necessary for obtaining Schwann cells [11, 12].

Nerve conduits represented an alternative to nerve regeneration through nerve autografts. The nerve tubes used for this purpose had different physical properties, according to the biomaterial and fabrication technique. The addition of Schwann cells, growth factors was used in order to improve nerve regeneration through these structures [13–15]. This combination of chemical, physical, and biological factors made the design of a nerve conduit into a complex process involving close collaboration of bioengineers, neuroscientists, and plastic surgeons.

The addition of Schwann cells as supporting cells was in favor for nerve regeneration of large nerve defects. However, the nerve matrix would not be formed within too large nerve defects. The addition of laminin, collagen, internal filaments, microtubules improved the nerve regeneration.

Mosahebi A *et al.* [16] used polyhydroxybutyrate conduits filled with alginate hydrogel, with cultured allogeneic or syngeneic Schwann cells. The conduits were used to bridge a 1 cm gap in the rat sciatic nerve, without the use of immunosuppressive therapy. Regeneration was examined after two, three, and six weeks with chemical staining and immunohistochemistry. Allogeneic grafts were rejected by six weeks. Nevertheless, both groups showed similar nerve regeneration.

The use of Schwann cell cultures in peripheral nerve regeneration [17] could be considered an option for the future. Schwann cell cultures used *in vitro* were able to support the reconstruction of peripheral nerve segments, which spindled the post grafting nerve regeneration. Experiments were carried out on rats in which peripheral nerves populated with cultured Schwann cells, were grafted onto the left optic nerve. Peripheral nerves were prepared from peroneal nerve sheaths populated with Schwann cells. High purity cell cultures were used for obtaining Schwann cells [17]. The lack of antigenicity in nerve sheaths prepared by freezing was documented. Nerve regeneration was shown in 3–4 weeks with immunohistochemistry, after the retina removal.

Mixed use of nerve allograft: Schwann cell cultures

Safe injection of Schwann cells into peripheral nerve allografts

The study was carried out in 2000 by Ogden MA *et al.* [18] on laboratory rats: Fischer rats served as recipient animals, and Buffalo rats provided tibial nerve allografts. Animals were divided into nine groups – Group I: rats receiving tibial nerve isografts, left untreated; Group II: rats injected with isogeneic Fischer Schwann cells; Group III: rats injected with placebo suspension; Group IV: rats receiving allografts, left untreated with Cyclosporin A; Group V: rats receiving isogenic Schwann cells; Group VI: rats receiving 2 mg/kg Cyclosporin A; Group VII: rats receiving 5 mg/kg Cyclosporin A; Group VIII: rats receiving 5 mg/kg Cyclosporin A with Schwann cells. Group IX of animals, harvested three days postoperatively, demonstrated no evidence of injection injury. The study showed that the injection of Schwann cells promoted the speed and accuracy of axonal regeneration in both isografts and allografts [18].

Peripheral nerve allografts usage avoided the donor sites morbidity, which was usually associated to grafts

harvesting, and offered an unlimited source of nerve grafts in order to reconstruct the defects resulted after complex, multiple traumas on large areas. The main goal was to promote regeneration through nerve allograft. Recent researches were focused [19] on Schwann cell autologous cultures combined with cold preserved allografts, association that decreased antigenicity and enhanced regeneration through allograft. Experimentally the method was tested on models in primates and in the pig. The results showed that Schwann cell model and cold denaturated allografts supported a regeneration similar to the autograft.

The evaluation of acellular nerve allografts seeded with Schwann cells in order to promote nerve regeneration after bridging the sciatic nerve defects in rat was communicated by Sun XH *et al.* (2009) [20]. Schwann cells were isolated from neonatal Wistar rats, and injected into acellular nerve allografts and co-cultured. Therefore, 24 Wistar rats were divided into three groups, with eight rats in each group: acellular nerve allografts seeded with Schwann cells group, acellular nerve allografts group and autografts group. In all groups, 1 cm long sciatic nerve gaps were created, grafted with the respective materials. Examinations of nerve regeneration were performed after 12 weeks by electron microscopy, electrophysiological methods, and then statistically analyzed. In conclusion, this study showed that acellular nerve allografts seeded with Schwann cells could improve nerve regeneration and functional recovery after bridging the sciatic nerve gap of the rats, offering a novel approach in the reconstruction of peripheral nerve defects. Positive results were materialized by the greater number of nerve fibers regenerated, by myelin sheath thickness and by myelinated fibers/total nerves (%), all results being higher in both autograft group and nerve allografts seeded with Schwann cells group [20].

Otherwise, Schwann cells represented the most antigenic component of peripheral nerve allograft. Tisack AM *et al.* (2008) [21] showed that the antigenic response of nerve allograft could be influenced by administrating MR1 antibodies. The administration of antibodies while performing the allograft decreased the immune response to Schwann cells. Therefore, functional results of the procedure in rats indicated an enhanced muscle contractility following nerve allograft in rats treated with MR. These data were indicative of a permissive state, which allowed migration of autogenous Schwann cells into the nerve graft.

Stem cells differentiation into Schwann cells

Prolonged denervation of Schwann cells seemed to be critical, as they became non-reactive to nerve axon regeneration. Good quality nerve regeneration was noticed where denervated Schwann cells had been replaced by healthy Schwann cells provided by a secondary nerve. Experimentally, peripheral nerve repair could be enhanced by Schwann cell transplantation, but this clinical application was limited by the donor site morbidity, and the low rate of cell differentiation–multiplication. Thus, the importance of research in stem cells therapy appeared and developed over time [22, 23].

Research in using cell precursors provided by skin cell was documented by Walsh S and Midha R in 2009. The great advantage of using skin derivatives consisted of its accessibility. Therefore, these stem cells survived and myelinated within the nerve, providing Schwann-like cells [22, 24].

Kingham PJ *et al.* [25] investigated whether adult stem cells, isolated from adipose tissue, could be differentiated into functional Schwann cells. Mesenchymal cells were isolated from enzymatic digested rat visceral fat, which further adopted a spindle-like morphology similar to Schwann cells, under the influence of glial growth factors. These results indicated that adipose tissue contained a pool of regenerative stem cells, which could be differentiated to Schwann cells, in the benefit of treating peripheral nerve injuries.

In using peripheral nerve allografts, the morbidity risk associated to the donor sites is avoided, and therefore an unlimited source of nerve grafts is provided in order to reconstruct the nerve defects resulted from complex, multiple traumas extended in large areas. The main goal is to promote regeneration through nervous allograft (nerve allograft and/or Schwann cell culture).

Although nerve regeneration can be experimentally achieved and/or enhanced by Schwann cells transplantation, this clinical application is limited, taking into account the donor site morbidity, and the low rate of cell differentiation-multiplication. Kingham RJ *et al.* investigated adult stem cells isolated from adipose tissue, and their differentiation into functional Schwann cells [25]. Mesenchymal cells, isolated from enzymatic digested rat visceral fat, further treated with a mixture of glial growth factors, adopted a spindle-like morphology similar to Schwann cells. These findings showed that adipose tissue contained a pool of regenerative stem cells, which could be differentiated to a Schwann cell phenotype, and could be of benefit in treating peripheral nerve injuries. Therefore, the laboratory findings advanced a very exciting perspective for nerve cell culture, and high suitable precursors, in peripheral nerve reconstruction.

Schwann cell cultures usefulness was shown as appropriate for: cell culture usage in peripheral nerve regeneration, conducted injuries in the central nervous system, studies of Schwann cells behavior, and Schwann cells–axons association induced by nerve injury.

Nevertheless, practically whether the cell culture supported the nerve regeneration, the results were not evenly positive, the success rate remaining still low. Although seemingly the method supported the peripheral regeneration without immunosuppressant secondary effects, the results being inferior to allografts subjected to denaturation and temporary immunosuppression.

However, the advances concerning *in vitro* cell harvesting technology and growth/regeneration factors represent new expectations regarding the clinical

availability of the methods involving Schwann cells and stem cells. In this respect, latest data and findings are to be mentioned. Thus, Vogt P *et al.* [26] presented surprising findings concerning neuronal implants comprising fibers made from natural or synthetic spider silk. They showed that implants allowed rapid nerve regeneration through these functional tubular sheaths. Neural implant properties allowed restoring stimulus conduction, being used in bridging defects in motor nerves over a distance of 2 cm to 4.5 cm or more. Nerve regeneration could be detected within a period of 10 days. Histological, there could be noticed that the implant was bridged by Schwann cells. By extending this method clinically, peripheral nerve reconstruction could be dramatically changed in the future [26].

Conclusions and future perspectives

In this article, the authors intended to demonstrate the necessity of expanding the conventional regeneration of peripheral nerves using alternative therapies. Schwann cells showed to be key factors in distal nerve regeneration by replacing degenerated host cells with exogenous cells, in favor for regeneration. Different sources of stem cells or precursors were identified as precursors for Schwann cells (skin, bone marrow, adipose tissue) [1].

Current techniques in tissue engineering may grant for a choice in peripheral nerves reconstructive strategies. Schwann cells are decisive in peripheral nerve regeneration. Therefore, Schwann cell cultures should be taken into account as possible choices in large peripheral nerve or spinal cord defects reconstruction. Furthermore, Schwann cells availability in peripheral nerves autogratfs offer a special benefit in supporting the nerve autogenous transplant. However, in using peripheral nerve allografts, donor areas morbidity related to autografts harvesting is avoided, and thus an unlimited source of nerve grafts is provided in order to reconstruct the defects resulted from complex, multiple traumas extended on large areas.

Allogeneic Schwann cell transplantation is to be considered in the reconstruction without any delay of peripheral nerve defects. As the association of nerve allograft injected with Schwann cells offers a tremendous support in nerve regeneration (in terms of speed and accuracy), this technique becomes similar to the nerve autografting.

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