PERSPECTIVE

Tubular conduits, cell-based therapy and exercise to improve peripheral nerve regeneration

Peripheral nerve injuries (PNI) are a major clinical problem. In general, PNI results from motor vehicle accidents, lacerations with sharp objects, penetrating trauma (gunshot wounds) and stretching or crushing trauma and fractures. It is estimated that PNI occur in 2.8% of trauma patients and this number reaches 5% if plexus and root lesions are included. However, due to lack of recent epidemiological studies, these data probably underestimate the actual number of nerve injuries.

Compared to the central nervous system, peripheral axons have the ability to regenerate. But, in human patients with injuries in the brachial or lumbosacral plexuses, this regenerative capacity is often incomplete, leaving frustrating functional deficits (Kehoe et al., 2012). This occurs particularly because the regenerative potential of cell bodies and the plasticity of Schwann cells (SCs) decline over time (Mirsky et al., 2002). Different animal studies have shown that prompt reinnervation of the end organ is the main determinant of a satisfactory functional recovery. It is well known that in human clinical set there is a delay in repairing an injured nerve and this delay can cause changes that make nerve fibers unable to regenerate, and the muscles undergo atrophy and lose the ability to be reinnervated (Martinez et al., 2014).

Take into account these data, researchers are constantly trying to develop strategies to increase the therapeutic success rate of regeneration and functional recovery. These techniques should be able to maintain the nerve regenerative capacity for long periods and to reduce the time of reinnervation of the target organ (Pereira Lopes at al., 2010). Thus, the combination of molecular approaches with novel forms of surgical repair followed by therapies which preserve muscle trophism is extremely necessary. For example, the association of cell-based therapies that improve axon regrowth with biodegradable conduits that bridge the gap after an injury, has already been proved to be an effective combination to promote nerve regeneration (Oliveira et al., 2010). Using this knowledge, we hypothesized that the addition of exercise therapy could be an interesting choice of a post intervention that may promote several systemic and local benefits, as well as potentiate the previously achieved effects. Thus, in our study, we chose to combine surgical repair with a tubular conduit, grafts of cultured Schwann cells, and treadmill training (**Figure 1**) (Goulart et al., 2014).

The development of an artificial nerve conduit as a bridge to guide the growing axons between the injured nerve stumps is a current experimental alternative to treat large peripheral nerve injuries. Pre-clinical animal models are extremely vital to test the effectiveness of nerve conduits prior to its use in the clinical practice. The ideal guidance conduit must be biocompatible and biodegradable, to avoid an inflammatory response and the need for a second

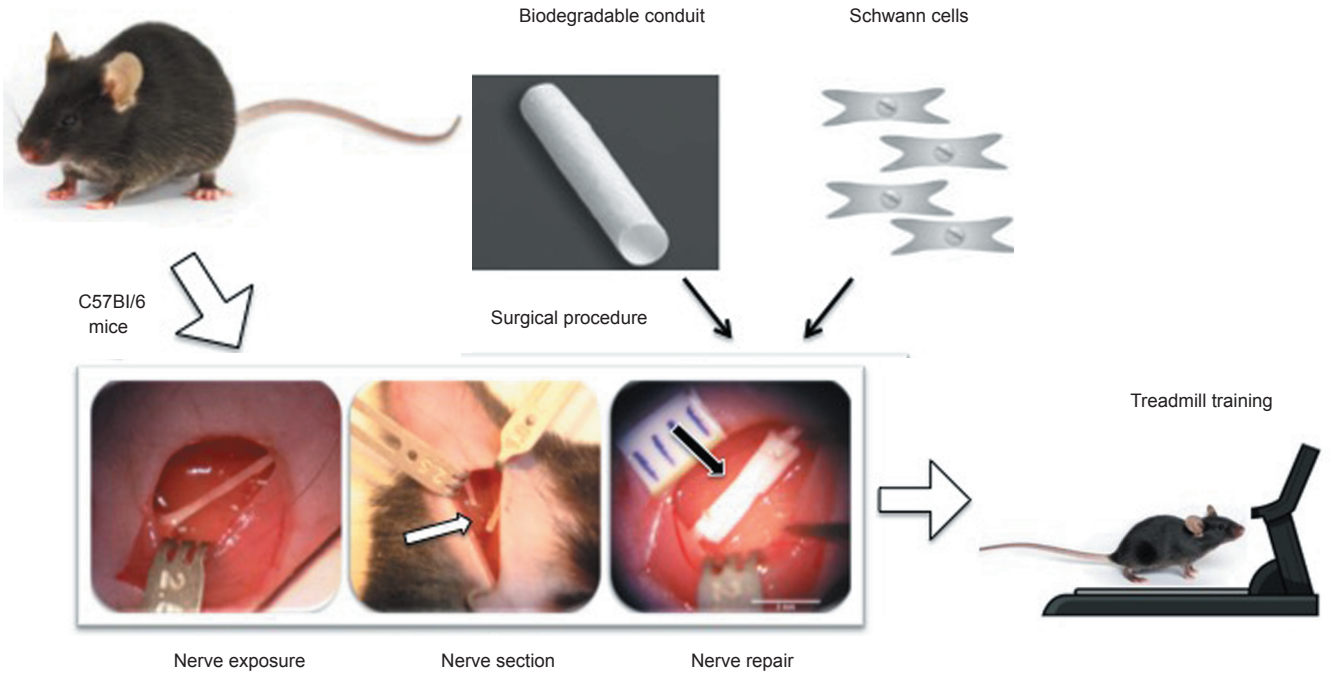

Figure 1 Schematic view of surgical repair using a tubular conduit with grafts of cultured Schwann cells and exercise training.
C57Bl/6 mice suffered an incision in the skin and the sciatic nerve was exposed; the sciatic nerve was transected (white arrow); biodegradable conduit was sutured into nerve stumps and Schwann cells were injected into the conduit (black arrow). Treadmill training started 3 days after injury, 3 times a week, during 8 weeks. Scale bar: 3 mm.

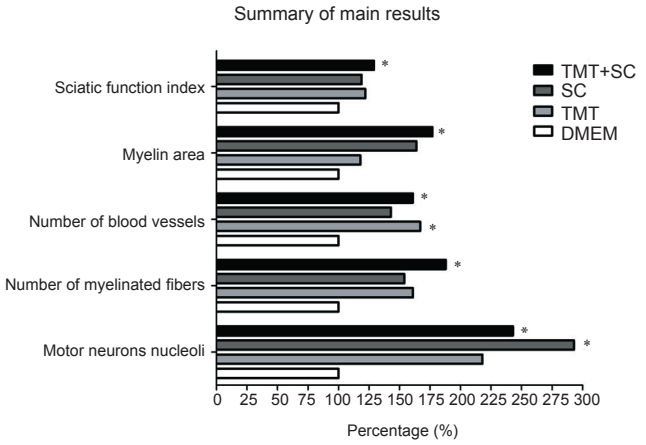

Figure 2 Data graph shows the percentage of improvement of analyzed parameters.
DMEM group average was considered as 100% and the treated groups were analyzed regarding this. Comparison shows statistically significant differences between the treated groups and DMEM (*$P < 0.05$).
TMT: Treadmill training; SC: Schwann cell; DMEM: Dulbecco's modified Eagle's medium.

surgical procedure. The device should act as a platform for Schwann cell migration and elongation of extending growth cones, possess an adequate strength and elasticity to withstand the muscles movement without collapsing and must be semipermeable to allow the diffusion of oxygen and nutrients and retain secreted neurotrophic factors (Kehoe et al., 2012).

According to Kehoe et al. (2012), the US Food and Drug Administration (FDA) has already approved different types of nerve guidance conduits for clinical use in peripheral nerve repair. In general, resorbable conduits offer greater advantages when compared to non-resorbable devices. The non-resorbable nature of some materials results in nerve compression and tension after nerve regeneration process has occurred. Among the resorbable materials commonly used in the manufacture of conduits are the collagen and poly-caprolactone (PCL). Collagen is a major component of extracellular matrix and has widespread use as a biological material; however, due to its low processing cost and ease of fabrication, PCL has recently gained considerable interest in the research field (Kehoe et al., 2012).

The tubular conduits are employed in combination with a number of guidance cues, to create a more permissive microenvironment. Examples of molecular and cell therapies include Schwann cells, stem cells, gene therapy, genetically modified cells and neurotrophic factors. In human clinical set, tubular conduits are only utilized for repair of thin digital nerves (Martinez et al., 2014).

In addition to the tubular conduit, cell-based therapies represent a new approach to treat peripheral nerve injuries. Among all the tested cell types, mesenchymal stem cells (MSCs) and SCs exhibit the most promising results (Oliveira et al., 2010; Goulart et al., 2014). MSCs act on nerve regeneration mainly by paracrine or immunomodulatory effects and they are strongly correlated with the production of neutrophic substances (Martinez et al, 2014). SCs are the myelin forming cells in the peripheral nerve system. They are also renowned for their plasticity and possess the remarkable ability to support cells during peripheral nerve regeneration (Mirsky et al., 2002; Pereira Lopes et al., 2010). It is thought that the beginning of the regenerative process in the peripheral nervous system is due to the dramatic regressive response of SCs to loss axon contact in damaged nerves, together with the autocrine mechanisms that allow SCs to survive in the absence of axonal contact. Furthermore, SCs are also able to secrete trophic factors, which are crucial for a successful regenerative process (Mirsky et al., 2002).

Exercise training has been proved to promote neuroplasticity, neurogenesis and neuroprotection, beyond learning and cognition (Bobinski et al., 2011). Long-term aerobic exercise can afford some neuroprotection to the peripheral nerves by preserving SCs and diminishing oxidative stress injuries. Low intensity aerobic exercise is also capable of reducing neuropathic pain by decreasing mechanical hypersensitivity and proinflammatory cytokine levels (Bobinski et al., 2011). It is also known that exercise can improve motor function after clinical and experimental peripheral nerve injury, and is helpful to treat loss of sensory function (Ilha et al., 2008). While the beneficial effects of exercise on axonal regeneration are well established, little information is available regarding the mechanisms by which physical activity exerts its effects.

To fill this lacuna, the aim of our study was to test the effectiveness of combining SC transplantation into a biodegradable conduit, with treadmill training as a therapeutic strategy to improve the outcome of repair after mouse sciatic nerve transection.

For our study, SCs were obtained from C57Bl/6 mice expressing green fluorescent protein (GFP^+ mice), according to the protocol described by Goulart et al. (2014). The surgical procedure used was sciatic nerve transection and tubulization with two types of conduits: polycaprolactone and collagen (Oliveira et al., 2010; Pereira-Lopes et al., 2010; Tonda-Turo et al., 2011). The conduit was grafted with SCs in DMEM (3×10^5/2 μL). Treadmill training started on the third day after sciatic nerve injury and repair, mice were trained at a belt speed of 10 m/min, during two 30 minutes exercise periods with a 10 minutes rest period between, 3 days per week, as described previously, with a few modifications (Udina et al., 2011).

Our results showed that the combined therapies were able to accelerate the sciatic nerve regenerative process, since animals treated with the three techniques presented a better functional outcome, indicated by the significant difference in the sciatic functional index at 7 days after injury. These results showed a clear correlation with the morphological analyses that exhibited an increased number of myelinated fibers and myelin area in the same group of animals (**Figure 2**).

In order to investigate if the regenerating nerve was capable of reaching his target organ, we also observed the morphology of neuromuscular junctions. In our findings, it

was possible to see a better structural organization of motor-nerve terminals, similar to a normal animal, in the group treated with combined therapies. This group also presented a better apposition of the motor nerve terminal in relation to the endplate, which suggested a more efficient reinnervation of the gastrocnemius muscle.

After an injury, nerve regeneration is enhanced by different neurotrophic factors, which includes nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), insulin-like growth factor (IGF), ciliary neurotrophic factor (CNTF), basic fibroblast growth factor (FGF-2), glial cell line-derived neurotrophic factor (GDNF) and others. In general, these neurotrophic factors are secreted molecules that play an important role in the development, function and plasticity of the nervous system. After an injury, they are also responsible for regulating neuronal survival and promoting axonal and neurite regeneration and reinnervation of target organs. We believe that the regenerative capacity of a nerve can be enhanced through the joint expression of different secreted neurotrophic factors. Therefore, as both exercise and cells were described for secrete trophic factors, we observed that SCs and treadmill training treatment increased the expression of neurotrophins in the sciatic nerve (BDNF, NGF and NT-4), in the dorsal root ganglia (BDNF, NGF, NT-3 and NT-4) and in the spinal cord (NT-4) (Goulart et al., 2014). These results may explain the greater degree of fiber myelination observed in the group treated with combined therapies. It is very likely that there is a complex interaction between regenerating axons and the regenerative environment. A more complete understanding of the essential role of each of these molecules in axonal regeneration will contribute to designing more efficient therapies for human injuries.

In conclusion, the combination of therapeutic strategies can significantly improve functional and morphological recovery. Moreover, transplanted SCs can be functionally incorporated into the regenerated tissue and have the capacity to secrete neurotrophic factors. These factors, either induced by physical exercise or directly released by transplanted SCs, are increased by the combination of therapies, most likely underlying the benefit of the association.

A limiting downside about the use of SCs as cell therapy in humans would be the need to sacrifice a healthy nerve in order to obtain the cells, plus the time necessary to harvest and expand these cells until reaching a number sufficient to transplant. Nevertheless, a great alternative source for SCs would be the use of stem cells, for example bone marrow stromal cells, which were previously described to express S100, a marker for SCs, indicating that these cells were able to survive and differentiate into a SC-like phenotype.

Despite the fact that it is still necessary to further investigate the mechanisms of the effect of combined therapies on axon regeneration, it is expected that these combinations of strategies will not only generate promising animal data, but will also play a role in a multi-factorial approach that is capable of bridging PNI. We strongly suggest that only those strategies that connect such multi-disciplinary approaches will be able to reach the optimal level of functional recovery for patients after a traumatic PNI.

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