PERSPECTIVES

Evaluating nerve guidance conduits for peripheral nerve injuries: a novel normalization method

The peripheral nervous system (PNS) is composed of the nerves and ganglia outside of the brain and spinal cord whose primary function is to connect the central nervous system to the limbs and organs. A peripheral nerve injury (PNI) is damage to the nerves and/or its surrounding tissue. These injuries can affect up to 5% of patients that are hospitalized for trauma (Taylor et al., 2008) and over 50,000 surgical repair procedures are performed annually in the United States alone (Evans, 2001).

The current gold standard of repair for injuries > 4 mm is an autograft. However, this method is limited due to the scarcity of donor nerves, potential donor site morbidity, differences in nerve diameter between the donor and grafting sites, as well as development of a painful neuroma (Mackinnon et al., 1990). A viable alternative is nerve guidance conduits (NGC), which are tubular guidance channels, which have been studied for a number of decades (Wang et al., 2011). NGCs have the potential to match the performance of autografts in terms of nerve regeneration, if developed with the optimal combination of haptotactic and chemotactic cues. While many novel conduits have been tested, only a few have been approved by the FDA, such as Neurotube®, NeuraGen®, Neuroflex™, NeuroMatrix™, AxoGuard™, and Neurolac® (Kehoe et al., 2012).

The continued challenge within tissue engineering has been to optimize the haptotactic and chemotactic parameters related to the design of an NGC to develop the most efficient product. Haptotactic cues are contact mediated cues that aid the growth cones of axons in reaching their targets. Examples include extracellular matrix (ECM) proteins and cell adhesion molecules. Chemotactic cues are diffusible cues such as growth factors which promote nerve regeneration and the survival, differentiation, and growth of neurons. Additionally cells can be coupled with haptotactic and chemotactic cues to enhance nerve regeneration (Yu et al., 2008). The traditional approach to optimizing an NGC is to conduct *in vitro* studies followed by *in vivo* studies. Researchers measure an array of parameters from *in vivo* to assess the quality of nerve regeneration and in turn the effectiveness of NGCs in bridging conduits. There are a number of parameters measured in *in vivo* studies: sciatic functional index, axon count and density, compound muscle action potential, and nerve conduction velocity (NCV) to name a few. This approach requires time, resources and can delay the commercialization of products. A drawback in the development of NGCs is the inability to compare the performance of NGCs for different gap lengths and account for different materials and enhancement factors.

In our approach to address the need for a normalization standard, we developed the relative regeneration ratio (RRR) which is based on an autograft's NCV (Chang et al., 2013). The objective of the RRR is to fulfill the unmet need of being able to compare NGC performance while accounting for variation in gap lengths, additives, and recovery time. The RRR uses the line of best fit to normalize the gap length and nerve conduction velocity data of an autograft as shown in **Figure 1**.

The RRR evaluates the performance of a NGC in relative to the autograft. The line of best fit is used for the clarification of the concept and not to depict a trend of regeneration. A RRR value of 1 means that an experimental conduit is just as good as an autograft, below 1 means the conduit is worse, and higher than 1 means the conduit is better than an autograft. By using RRR, researchers can compare the performance of NGCs, account for materials, enhancement factors, and as variations in gap length. A significant aspect of this approach is that it can account for recovery time in comparison of NGC performance, a feature not possible with any other normalization standard. We validated the RRR normalization standard using data collected from 10 and 15 mm gap length studies conducted for 4 and 8 weeks in a rat sciatic nerve injury model.

Researchers have traditionally focused their efforts in mimicking the autograft in terms of NGC structure, inclusion of chemotactic and haptotactic factors, as well as cells present in autografts. They have also focused on the methods that are optimal for delivering and releasing growth factors and ECM proteins. The studies that are conducted in regard to NGCs are based on the current understanding of the mechanism of nerve regeneration.

Autografts are excised nerve trunks so their structure generally remains intact. Conduits are at a disadvantage in comparison to the autograft, so even the best conduits' RRR values are below the level of autograft at week 4 and week 8, but once conduits overcome this "handicap," the benefits of their materials and structure begin to have a greater effect, thus increasing their regeneration rate and RRR value. Also, an autograft's "head-start" allows it to reach its maximum regeneration potential earlier than a conduit. As such when an autograft nears its maximum recovery point and begins reaching its plateau in regeneration, the conduits are just reaching a level where they can regenerate quickly. Understandably, the conduits begin increasing their regeneration rate relative to the autograft because the autografts are slowing down. Using RRR researchers can be one step closer to developing the optimal conduit for PNI or potentially have a technique that can be used to develop the optimal conduit for a specific PNI. RRR is capable of assessing how close an NGC is in terms of functional performance to an autograft which can allow researchers to adjust their haptotactic and chemotactic cues to mimic the performance of an autograft. Since the RRR is based on a measure of functionality its application in comparison of NGC performance can improve the understanding of the mechanism of nerve regeneration, such as the significance of contact guidance theory.

The growing number of global PNI cases triggers the need for a technique to develop optimal NGCs. The goal of any

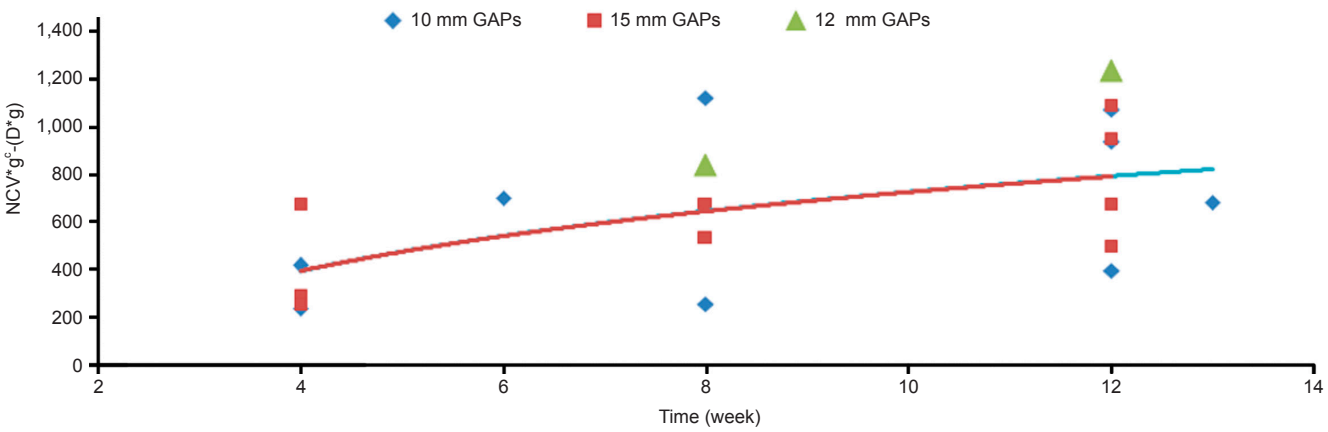

Figure 1 Graph of the autograft normalization function for all available autograft data with time.
Reprinted with permission from (Chang et al., 2013) ©2013 Wiley. The 12 mm autografted data is not significantly different from the 10-mm and 15-mm data ($P > 0.05$). This indicates that the normalization function successfully normalized the data.

NGC is to provide complete recovery to an injured nerve. Normalization standards such as RRR can allow this goal to be achieved by providing a deeper insight into the mechanism of nerve regeneration. The traditional burdens, in vitro and *in vivo* testing, can be alleviated if RRR is used in the form of a prediction model. This can allow researchers to predict the outcome of combining chemotactic and haptotactic cues to reduce the time and resources spent on experiments to investigate such an outcome.

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