The sciatic nerve injury model in pre-clinical research

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Abstract

In the pre-clinical view, the study of peripheral nerve repair and regeneration still needs to be carried out in animal models due to the structural complexity of this organ which can be only partly simulated in vitro. The far most used experimental model is based on the injury of the sciatic nerve, the largest nerve trunk in mammals. In this paper, the potential application of the sciatic nerve injury model in pre-clinical research is critically reviewed. This paper is aimed at helping researchers in properly employing this in vivo model for the study of nerve repair and regeneration as well as interpreting the results in a clinical translation perspective.

Keywords: Axonal regeneration; Nerve reconstruction; Nerve fibers; Functional recovery; Animal models

1. Introduction

Because of their spread distribution throughout the all body, peripheral nerves are particularly subject to injuries mainly due to traumatic, e.g. work accidents, or iatrogenic, e.g. for tumor excision lesion (Evans, 2001; Siemionow and Brzezicki, 2009; Isaacs, 2013). Although usually not threatening the patient's life, nerve injuries represent a heavy social burden in terms of both long term disability and economic costs (Asplund et al., 2009; Rosberg et al., 2013). For this reason, growing efforts are dedicated to the development of effective treatment for peripheral nerve injuries which increase tissue regeneration and functional recovery and might be eventually translated to the patients for improving the clinical outcome (Tos et al., 2013, Griffin et al., 2013).

This body of pre-clinical research is mainly carried out in animal models since, so far, in vitro investigation of nerve regeneration is very limited due to the structural complexity of this organ which can hardly be reproduced in vitro (Geuna et al., 2009). The far most used experimental paradigm for the pre-clinical investigation of peripheral nerve regeneration is represented by the sciatic nerve injury (SNI) model (Sironen et al., 1996; Beer et al., 2001; Varejão et al., 2004, Nichols et al., 2005; Savastano et al., 2014). Among the various reasons that might explain the preponderancy of SNI employment, two are the most important: (i) the large size of the sciatic nerve which facilitates surgery; (ii) the easy surgical access; (iii) the sought for data that can be comparable with previous studies, the very large majority of which have been carried out using the SNI model.

Due to the enormous number of experimental papers reporting data obtained with SNI model, a comprehensive review would be almost impossible and, probably, not so useful for researchers. By contrast, the aim of this paper is to overview a selection of relevant papers with the goal of providing the reader with some useful indications about the potentiality of employment of the SNI model as well as some methodological information that might help researchers in critically interpreting the results in a translational perspective.

2. Compression lesions

Experimental models based on the mechanical compression of the sciatic nerve have been widely used in experimental research in order to investigate the changes occurring to the nerve, proximal and distal to the lesion, as well as to the other central (e.g. neuronal cell bodies) and distal (e.g. muscles) anatomical structures. Sciatic nerve compression can be obtained by either ligation or crush of the epineurium. While ligation, that can be transiently applied and is used of the study of neuropathic pain (Challa, 2014), mainly induces functional changes only and it falls thus outside of the topic of this review, the crush lesion causes permanent anatomical damage and is one of the most used experimental models for the study of nerve repair and regeneration in the pre-clinical perspective (Bridge et al., 1994; Savastano et al., 2014).

Various methods have been devised for producing the crush injury, including various surgical instruments (Chen et al., 1992; Kingery et al., 1994, Savastano et al., 2014) and compression devices (Navarro and Kennedy, 1989; Radevik and Lundborg, 1997; Oliveira et al., 2001; Srikcioglu et al., 2007). The compression is applied with the goal of interrupting the continuity of all axons (axonotmesis) without interruption of the connective scaffold of the nerve (especially the epineurium) and thus without losing continuity of the nerve trunk. Therefore, the nerve segments proximal and distal to the lesion site remain connected allowing severed axons to regrowth along an optimal regenerating pathway (the distal Wallerian regeneration environment) and reach original innervation targets (Geuna et al., 2009).

In 2001, Beer et al. described a non-serrated clamp aimed at exerting a standardized pressure to the nerve. This device has proven to be reproducible in in different animal species (Beer et al., 2001; Varejao et al., 2004) and its use is spreading among peripheral nerve regeneration researchers.

Independently of the procedure, the crush lesion has the main advantage to do not require microsurgical skills. Yet, inter-individual variability in tissue regeneration as well as in functional recovery is limited. These features make the sciatic nerve crush injury model particularly suitable for

the study of the biology of peripheral nerve regeneration as well as the treatment strategies to improve it. In fact, its high reproducibility makes easier the identification of the changes occurring not only to the entire tissue but also at the cellular and molecular level (Chen et al., 2008; Tooth et al., 2008; Lou et al., 2012; Long et al., 2013; Wright et al., 2014). Yet, high reproducibility of the lesion makes this experimental model also particularly suitable for investigating regeneration-related time course changes (De Leon et al., 1991; Gupta and Channual, 2006; Sta et al., 2014). Finally, changes in the outcome of nerve regeneration after a crush injury of the sciatic nerve might be used as a pre-clinical end-point predictor of the effectiveness of a therapeutic agent and/or tissue engineering strategy on nerve regeneration (Fleming et al, 2007; Amado et al., 2008; Baptista et al., 2008; Gigo-Benato et al., 2010; Dadon-Nachum et al., 2011; Kilic et al., 2013; Wang et al., 2014).

3. Transection lesions

Although the experimental model based on the sciatic nerve crush injury has several advantages in terms of feasibility and reproducibility, its translational potential is limited for two main reasons. First, most surgically relevant nerve lesions in human patients are characterized by at least partial transection/laceration of the nerve. Second, crush lesions in patients have a different clinical history in comparison to experimental crush lesions in laboratory animals, namely spontaneous axon regeneration observed in laboratory animals does not often occur in humans due to frequent extensive fibrosis at the lesion site; thus, in many cases, crush lesions in patients require surgery for removing the damaged tissue and replace it with a conduit (Tos et al., 2012).

For this reason, the translation to the clinics of an innovative nerve repair and regeneration treatment need in most cases to be validated using an adequate nerve transection experimental model that mimics the relevant human clinical condition as outlined in the following paragraphs.

3.1. Chronic denervation

The most severe clinical condition that can be met in a patient is the chronic denervation, i.e. a complete transection of a nerve not followed by reconstruction of the nerve continuity (e.g. because of proximal nerve stump's avulsion or multiple nerve defects with insufficient availability of autograft tissue). This condition usually occurs as a consequence of severe and multiple nerve traumas, e.g. brachial plexus injury, and induces the permanent disconnection between neurons and the respective distal nerve targets with definitive loose of sensory and/or motor function.

Chronic denervation can be reproduced by complete transection of the sciatic nerve not followed by its surgical reconstruction. Since in laboratory animals axonal regeneration is more pronounced than in humans and can occur spontaneously after complete transection even in absence of nerve repair, particular attention should be paid in avoiding this occurrence by turning the proximal nerve stump and suturing it to a neighboring tissue (e.g. a muscle).

Complete transection not followed by surgical repair of the sciatic nerve induces the loose of motor function of posterior muscles of the thigh and of all muscles below the knee. Yet, sciatic nerve transection induces also loose of sensory function of large areas of the hindlimb including most of the foot, a condition that is at the basis of the progressive auto-mutilation observed in the post-operative (see # 7.6).

Therefore, while SNI is not suitable to study the effects of chronic denervation on the sensory function, this experimental model is, by contrast, a very useful approach for the study of denervation of the distal nerve trunk and the skeletal muscles as well as the sought of effective strategies to prevent it (Russo et al., 2007; Saito et al., 2009; Karsidag et al., 2012; Moimas et al., 2013; Blom et al., 2014).

Since changes in both distal nerve segment and target muscle occur very early, the model can be used to study both early and late events post-denervation. As regards the time course of early post-denervation changes, a 1-month delay is a good option for both nerve and muscle. In both organs, in fact, degeneration is very active in the first post-injury days and at 1-month atrhophy has already well established. Afterwards, the progress of degeneration is much slower and it is difficult to determine

timepoint for long term denervation. Most authors adopt a delay ranging from 3 to 6 months after injury.

3.2. Direct suture repair (end-to-end neurorrhaphy)

In many cases, however, nerve transection in humans can be treated surgically and, if no substance loss occurs, the direct suturing of the two nerve stumps restores nerve continuity and allows axonal regeneration along the distal target towards the original motor and sensory targets (Geuna et al., 2009). Although it is already a widely established surgical technique, the SNI model can still be a useful tool for investigating end-to-end neurorrhaphy in a pre-clinical view with several main scopes, such as exploring alternative methods for connecting the nerve stumps, especially glues (Felix et al., 2013) and identifying effective strategies for reducing post-surgical scar-tissue formation at the suture site (Que et al., 2013).

3.3. Nerve graft reconstruction of sciatic nerve defects

The loose of substance after a nerve trauma requires that the direct suturing for reconnecting the two stumps is made under tension, a condition which might limit regeneration and functional recovery (Battiston et al., 2009; Siemionow and Brzezicki, 2009). Therefore, today nerve defects are treated by the interposition of an autograft, a technique that has been introduced in in the 70th (Berger and Millesi, 1979). Autograft repair of the sciatic nerve is still today widely used in pre-clinical research since it represents the benchmark condition toward which alternative types of nerve guides are tested (Siemionow and Brzezicki, 2009; Griffin et al., 2013). However, it should be clearly pointed out that the technique used for autograft sciatic repair in laboratory animals (i.e. the removal of a nerve segment followed by its immediate re-implant with or without 180° rotation) significantly differs from the autograft technique used in patients (i.e. interfascicular nerve grafting of the damaged nerve using multiple segments of a sensory nerve, usually the sural one), a discrepancy that

should be always taken into consideration in the interpretation of the experimental results in a pre-clinical perspective.

More recently, experiments based on the SNI model have also provided the pre-clinical proof of concept that decellularized allografts can be a good alternative to gold standard autografts (Whitlock et al., 2009) providing the pre-clinical basis for the successful introduction of decellularized allografts to the clinics (Brooks et al., 2012).

3.4. Tubulization reconstruction of substance defects

Although autografts still remain the gold standard for nerve defect reconstruction, this technique may cause secondary damage; yet, autograft availability, in terms of length of graft material, may be insufficient in case of massive nerve injuries (Battiston et al., 2009; Siemionow and Brzezicki, 2009). Therefore, a number of alternatives, both biological or synthetic, have been assessed in a pre-clinical setting in order to substitute nerve autografts. Un-doubtfully, the search for alternatives to nerve autografts is the field where SNI model has seen most extensively employment. The SNI model has been used for testing conduits for nerve repair of both of biological and synthetic origin. As regards biological nerve guides, various autologous tissues have proven to be effective in repairing sciatic nerve gaps with performances, in terms of both histological and functional predictors of recovery, that were close to those obtainable with autografts (Chiu et al., 1982; Glasby et al., 1986; Geuna et al., 2000).

In spite of the effectiveness of some types of biological nerve guides, some of which have been successfully translated to the clinics (Chiu and Strauch, 1990; Pereira et al., 1991; Marcoccio and Vigasio 2010; Tos et al., 2012; Manoli et al., 2014), most research along the last 30 years has been dedicated to artificial scaffolds based on the recent advancements in bio-nanotechnologies. A number of innovative artificial nerve guides have been developed and this body of experimental research has been mainly based on experiments made using the SNI model aimed at comparing, in a pre-clinical view, the effectiveness of different types of scaffolds (Rodriguez et al., 2000; Varejao et

al., 2003; Dodla and Bellamkonda, 2008; Carriel et al., 2013; Haastert-Talini et al., 2013; Reid et al., 2013; Johansson and Dahlin, 2014).

4. Considerations about selection of the animal species

The SNI model has been used in number different animal species. The rat is by far the most used species (Angius et al., 2012) as shown by more than 13,000 entries obtainable in a PubMed query up to July 2014. When the same query is carried out for the mouse, the second most used animal species, the number of entries drops to about 3,500, while the third most widely used species for SNI model is the rabbit with about 1,500 entries. Whereas the rat clearly represent the species of choice for SCI studies, the use of the latter two animal models of SNI are appropriate when specific research goals are required, namely the mouse for the availability of genetically manipulated animals (Tos e tal., 2008, Willemen et al., 2010 in Savastano, Ronchi et al., 2010; Eijkelkamp 2010 in Savastano) or the rabbit in case of the study of devices that are too large in comparison to the rat sciatic nerve size, i.e. nerve prostheses longer than 1.5 cm (Geuna et al., 2004; Hsu et al., 2011; Gao et al., 2013).

Although the rat, mouse and rabbit are the most used species for SNI experimental investigation, several other mammals have also been used to the same end, including the mini-pig (Uranus et al., 2013), the guinea pig (Rao et al., 2001) the dog (Xue et al., 2012) and the cat (Sufan et al., 2001) due to their larger body size. Yet, also SNI in primates has been used for pre-clinical testing of nerve scaffolds (Archibald et al., 1991) due to its closer similarities with human patients. However, the growing ethical concerns about research on primates is strongly limiting the indication to their employment due to the relative evolutionary conservation of nerve injury and regeneration features in all mammal species.

Finally also non mammalian species has been used for SNI investigation, such as the chick () and the frog (Blanco et al., 1999). However, these studies should be regarded mainly for evolutionary investigation and not in the pre-clinical perspective due to the much more pronounced regeneration potential of non-mammalian species in comparison to mammals.

5. Methodological considerations

5.1. Surgery

Experimental surgery of to the sciatic nerve is relatively easy due to its large size (the largest nerve in mammals). The sciatic nerve is a mixed nerve (Schmalbruch, 1986) which originates from the lumbo-sacral plexus and ends at the knee level with its terminal division that is usually represented by a trifurcation: the tibial nerve (the biggest one) the common peroneal nerve and the sural nerve (Rupp et al., 2007b). However, there is a high anatomical variability in the number and site of origin of sciatic nerve terminal branches which should be always taken into consideration, especially in the identification of the lesion site.

A second methodological consideration about surgery is the maximum length of the sciatic nerve defect that, in the rat, should be limited to 1.5 cm. Although the bridging of gaps longer than 1.5 cm have been described in the rat (e.g. Geuna et al., 2000; Dodla and Bellamkonda, 2008), it is preferable to move to large animal models (e.g. rabbit or sheep) when long nerve prostheses have to be tested in vivo.

5.2. Functional assessment

Although the assessment of the functional outcome is the more important evaluation parameter in the pre-clinical perspective, most currently available methods for measuring functional recovery after SNI are characterized by a high degree of variability which, unfortunately, limits data interpretation.

As regards motor function recovery, the far most commonly used test is the calculation of the sciatic functional index (De Medinaceli et al., 1982; Varejao et al., 2001; Baptista et al., 2007). Although this method is the very popular in peripheral nerve regeneration research, its validity has been questioned (Varejao et al., 2004). Therefore, more recently, the availability of high-performing video cameras has allowed the development of more reliable computerized gait analysis system based

on the video recording of the animals (Pereira et al., 2006; Bozkurt 2008a,b; Costa et al., 2009). Finally, it has also been suggested that the BBB scale, a method commonly used for the study of spinal cord injury and regeneration (Basso et al., 1995), can be also a valuable additional method for the assessment of sciatic nerve injury and regeneration too (Dinh et al., 2009). This method is based on a 21 point scale based on the analysis of specific components of functional behavior, such as the limb movement, paw placement/position and stepping. A score of 0 is given if there was no spontaneous hindlimb movement, a score of 21 indicates normal locomotion.

As regards sensory function recovery, several tests have been proposed. Among these, the withdrawal reflex latency test (using a hotplate) to assess nociceptive function (Masters et al. 1993). and the Von Frey test (Cobianchi et al., 2014).

5.2. Electrophysiological assessment

The electrophysiological assessment of the nerve recovery is the predictor of nerve regeneration which is closer to the direct assessment of the motor or sensory function. Therefore, the complexity and sometimes even the impossibility of a direct functional assessment in laboratory animals makes electrophysiology a precious tool in peripheral nerve regeneration research (Rupp et al., 2007a; Navarro and Udina, 2009). Being a mixed nerve, the electrophysiological assessment of the sciatic nerve can be carried out both for the efferent and afferent component.

Since recovery of motor function is the most relevant postoperative achievement that is sought in the pre-clinical perspective, the most used electrophysiological method is the recording of evoked compound muscle action potentials (CMAPs) after electrical stimulation proximal and distal to the lesion site (Navarro and Udina, 2009; Nijhuis et al., 2013). As regards the sensory component, the most used method for the electrophysiological assessment is the recording of the somato-sensory evoked potentials (SSEPs) (Navarro and Udina, 2009; Chow et al., 2012) although their employment in laboratory animals has been debated (Navarro and Udina, 2009). Finally, also electromyography

(EMG) has proven to be useful for the evaluation of muscle re-innervation after sciatic nerve repair (Gransbergen et al., 2000).

5.4. In vivo imaging

Recent advances in *in vivo* imaging techniques of tissues and organs have more and more expanded their use to small animals species. As regards SNI investigation, two methods are receiving growing interest for monitoring in vivo the nerve regeneration process: ultrasonography and magnetic resonance.

Ultrasound imaging has the advantage that can be obtained using relatively cheap instruments that, today, have reach a resolution level that allow to visualize very small soft structures such as peripheral nerves in laboratory animals (Kuffler, 2010). In expert hands, this technique can thus be used also in rat sciatic nerve to monitor the progression of nerve tissue regeneration, e.g. along a nerve prosthesis (Chen et al., 2014).

Magnetic resonance requires much more expensive devices that should adapted to the size animal species under investigation (Behr et al., 2009). However, if a dedicated facility is available, magnetic resonance imaging holds great expectations for in vivo SNI investigation in experimental models (Liao et al., 2012; Yamasaki et al., 2015).

5.5. Histology and histomorphometry

Histological examination of the nerve segment is one of the pillars of nerve repair and regeneration research and an essential complement to the functional and electrophysiological investigation techniques. Histology can give information not only on the presence of regenerated axons, but also the occurrence of inflammatory processes and fibrosis, both inside the nerve and outside it (perineurial adhesions) and of neuroma formation. Yet, if biomaterials are used to bridge nerve defects, histology can give important information also about degradation of the materials and the presence of foreign body reaction and granuloma. Many tissue processing and staining techniques

are available and it is far beyond the goals of this paper to revise that body of literatue (see Raimondo et al., 2009; Carriel et al., 2014). Anyway, histology is adopted by the large majority of authors with the goal of quantifying the number and size of regenerated nerve fibers and myelin sheath thickness.

To reach this goal, whereas several histochemical and immunohistochemical methods have proven to be useful (Di Scipio et al., 2008; Sinis et al., 2009; Carriel et al., 2014) , the far most used method is toluidine blue staining on semithin sections from osmium post-fixed and resin-embedded blocks (Raimondo et al., 2009). This method allows high resolution imaging of myelinated nerve fibers. It should be noted however, that the histological appearance of the sciatic nerve is very variable not only along it course (it is usually single fascicled at its origin, while it is divided in a variable number of fascicles along its distal part) but also at the same level when different animals are compared. This occurrence makes the morphological analysis of sciatic nerve regeneration particularly tricky especially when quantitative data about myelinated nerve fiber number and size are sought. For this reason, a rigorous randomization protocol should be adopted to avoid severe bias in the histomorphometrical data obtained. In addition, the irregular size and shape of nerve fibers makes it necessary the use of unbiased counting and measuring methods such as the unbiased counting frame and the 2D-disector methods (Larsen 1998; Kaplan et al., 2010).

Finally, it should be noted that even if high resolution light microscopy observation is adopted, myelinated nerve fibers with a diameter smaller than 2um might not be detectable resulting in a significant underestimation of the their total number especially in regenerated nerves (Ronchi et al., 2014).

5.6. Molecular biology

Also molecular biology techniques, especially PCR for RNA analysis and Western-blotting for protein analysis, can be used to study sciatic nerve regeneration. However, it should be noted that the nerve comprises many different cell types and thus protein and RNA data are often hard to be

interpreted. In addition, PCR analysis fails to reveal neuronal RNA that is mostly localized in the cell bodies.

5.7. Ethical issues: animal wellbeing

SNI induces significant limitations in the movements of the affected limb accompanied by loss diffuse of sensation, especially in the foot. Motor function loss, besides walking impairment, often induces muscle contractions (Dellon and Mackinnon 1989). On the other hand, the main drawback of sensory function loss is the progressive autotomy of variable degree, from simple nail lost to extensive mutilation of the entire foot (Krsljak and Stajcic, 2004).

If the experimental paradigm leads to fast nerve regeneration, e.g. the crush injury model, functional impairment is transitory with reduced discomfort to the animal. On the other hand, in an ethical view point, the impact of the functional impairment induced by SNI on animal wellbeing should be taken into serious consideration in case of experimental conditions that induce long term functional impairment, e.g. chronic denervation and/or complex reconstruction treatments which require a long-term regeneration process.

6. Discussion

The investigation of nerve repair and regeneration has a long history and still today represents, un-doubtfully, the most addressed issue in the study of the peripheral nervous system.

The growing ethical concerns regarding the use of animals in biomedical research and the progressive spread among the scientific community of the ‘‘Three Rs’’ concept (replacement, reduction, and refinement of animal studies) (Russell and Burch, 1992) pushes for an increase in the replacement of in vivo models with in vitro pre-clinical models which might mimic nerve regeneration processes avoiding the use of animals for testing new repair and regeneration strategies (Tos et al., 2009). However, although many immortalized and primary neuronal and glial cell lines (Moreno-Flores et al., 2006, Hara et al., 2008; Sak and Illes, 2005; Shastry et al., 2001; Trotter, 1993, De Paola et al., 2007, Scanlin et al., 2008) have been proposed for replacing in vivo pre-clinical models, their potential is still limited and, yet, their real capability of predicting nerve regeneration is questionable (Cirillo et al., 2014). The potential of the in vitro investigation of nerve regeneration may be higher if the culturing conditions mimic the 3D organization of the nerve. This can be obtained either by 3D co-cultures where the spatial organization of neuronal and glial cells is reproduced (Bozkurt et al., 2007; Fornaro et al., 2008, Gingras et al., 2008; Vyas et al., 2010; Siddique et al., 2014). However, 3D cell cultures have a high technical complexity which limits their use and, yet, their reliability as providers of pre-clinical endpoints in the translational perspective is not widely acknowledged and thus the use of in vivo pre-clinical models of nerve regeneration is still necessary.

As already outlined in this paper, the large majority of the in vivo animal studies have been carried out by inducing a lesion of the sciatic nerve. However, recently, experimental models based on the use of other nerves have been proposed, especially the median nerve because of the availability of a simple and reliable behavioral test, the grasping test (Lutz et al., 2000; Bontioti et al., 2003; Papalia et al., 2003; Galtrey and Fawcett, 2007; Sinis et al., 2006; Wang et al., 2008). In fact,

after the lesion of the median nerve animal welfare is preserved (Papalia et al., 2003) and the results are more likely to be translated to the clinical practice (the main goal of pre-clinical studies) since the large majority of surgeries of human nerves are performed in the upper limb. In addition, the grasping function requires a fine and skilled movement of the fingers that is quite similar between the mostly used laboratory animals (mice and rats) and humans (Whishaw et al., 1992). Finally, the median nerve is usually single fascicled in contrast to the multiple fascicles of the sciatic nerve, a histological feature which makes quantitative morphological analysis easier. However, a main limitation of the rat and mouse median nerve, in comparison to the sciatic nerve, is the small size which requires availability of high microsurgical skills for the epineurial suturing.

In conclusion, although alternatives are available, the sciatic nerve injury model still represent a widely used method for pre-clinical research on nerve repair and regeneration. Whereas a consensus appears to be needed in order to clearly define the adequate ambits of SNI model's employment and the minimal reporting standards when this model is used in a pre-clinical perspective, I wish to emphasize that, according to our present knowledge, it appears that there is no single experimental model of nerve injury and regeneration which is inherently superior to the others. Therefore, my suggestion is that researchers select the pre-clinical model which best fits their needs after a careful assessment of their specific requirements and expertise, knowing each model's advantages and limitations, and interpreting the results within those limitations, instead of hewing to a rigid point of view about which model is the best. In this view, the information provided in this review can be of help for researchers in properly employing this in vivo experimental model for the study of nerve repair and regeneration. Yet, this is aimed to help researchers in selecting the investigation methods which best fits with their study's goals as well as in adequately interpreting the results in a clinical translation perspective.

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References

Amado S, Simões MJ, Armada da Silva PAS, Luís AL, Shirosaki Y, Lopes MA, Santos JD, Fregnan F, Gambarotta G, Raimondo S, Fornaro M, Veloso AP, Varejão ASP, Maurício AC, Geuna S. Use of hybrid chitosan membranes and N1E-115 cells for promoting nerve regeneration in an axonotmesis rat model. Biomaterials, 2008, 29:4409-19.

Angius D, Wang H, Spinner RJ, Gutierrez-Cotto Y, Yaszemski MJ, Windebank AJ. A systematic review of animal models used to study nerve regeneration in tissue-engineered scaffolds. Biomaterials, 2012, 33:8034-9.

Archibald SJ, Krarup C, Shefner J, Li ST, Madison RD. A collagen-based nerve guide conduit for peripheral nerve repair: an electrophysiological study of nerve regeneration in rodents and nonhuman primates. J. Comp. Neurol., 1991, 306:685-96.

Asplund M, Nilsson M, Jacobsson A, von Holst H. Incidence of traumatic peripheral nerve injuries and amputations in Sweden between 1998 and 2006. Neuroepidemiology, 2009, 32:217-28.

Baptista AF, Gomes JR, Oliveira JT, Santos SM, Vannier-Santos MA, Martinez AM. A new approach to assess function after sciatic nerve lesion in the mouse - adaptation of the sciatic static index. J. Neurosci. Methods, 2007;161:259-64.

Baptista AF, Gomes JR, Oliveira JT, Santos SM, Vannier-Santos MA, Martinez AM. High- and low-frequency transcutaneous electrical nerve stimulation delay sciatic nerve regeneration after crush lesion in the mouse. J. Peripher. Nerv. Syst., 2008;13:71-80.

Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. J. Neurotrauma, 1995, 12:1-21.

Battiston B, Raimondo S, Tos P, Gaidano V, Audisio C, Scevola A, Perroteau I, Geuna S. Tissue engineering of peripheral nerves. Int. Rev. Neurobiol., 2009, 87:227-49.

Beer GM, Steurer J, Meyer VE. Standardizing nerve crushes with a non-serrated clamp. J. Reconstr. Microsurg., 2001, 17:531-34.

Behr B, Schnabel R, Mirastschijski U, Ibrahim B, Angenstein F, Schneider W. Magnetic resonance imaging monitoring of peripheral nerve regeneration following neurotmesis at 4.7 Tesla. Plast. Reconstr. Surg., 2009, 123:1778-88.

Berger A, Millesi H. Nerve grafting. Clin. Orthop. Relat. Res., 1978, 133:49-55.

Blanco RE, Rosado J, Padilla J, Del Cueto C. Ultrastructural studies of dorsal root axons regenerating through adult frog optic and sciatic nerves. Microsc. Res. Tech., 1999, 46:310-8.

Blom CL, Mårtensson LB, Dahlin LB. Nerve injury-induced c-Jun activation in Schwann cells is JNK independent. Biomed Res. Int., 2014, 2014:392971.

Bontioti E, Kanje M, Dahlin LB. Regeneration and functional recovery in the upper extremity of rats after various types of nerve injuries. J. Peripher. Nerv. Syst., 2003;8:159-68.

Bozkurt A, Brook GA, Moellers S, Lassner F, Sellhaus B, Weis J, Woeltje M, Tank J, Beckmann C, Fuchs P, Damink LO, Schügner F, Heschel I, Pallua N. In vitro assessment of axonal growth using dorsal root ganglia explants in a novel three-dimensional collagen matrix. Tissue Eng., 2007, 13:2971-9.

Bozkurt A, Deumens R, Scheffel J, O'Dey DM, Weis J, Joosten EA, Führmann T, Brook GA, Pallua N. CatWalk gait analysis in assessment of functional recovery after sciatic nerve injury. J. Neurosci. Methods, 2008a;173:91-8.

Bozkurt A, Tholl S, Wehner S, Tank J, Cortese M, O'Dey D, Deumens R, Lassner F, Schügner F, Gröger A, Smeets R, Brook G, Pallua N. Evaluation of functional nerve recovery with Visual-SSI-A novel computerized approach for the assessment of the static sciatic index (SSI). J. Neurosci. Methods, 2008b;170:117-22.

Bridge PM, Ball DJ, Mackinnon SE, Nakao Y, Brandt K, Hunter DA, Hertl C. Nerve crush injuries--a model for axonotmesis. Exp. Neurol., 1994;127:284-90.

Brooks DN, Weber RV, Chao JD, Rinker BD, Zoldos J, Robichaux MR, Ruggeri SB, Anderson KA, Bonatz EE, Wisotsky SM, Cho MS, Wilson C, Cooper EO, Ingari JV, Safa B, Parrett BM, Buncke GM. Processed nerve allografts for peripheral nerve reconstruction: a multicenter study

of utilization and outcomes in sensory, mixed, and motor nerve reconstructions. Microsurgery, 2012, 32:1-14.

Carriel V, Garrido-Gómez J, Hernández-Cortés P, Garzón I, García-García S, Sáez-Moreno JA, Del Carmen Sánchez-Quevedo M, Campos A, Alaminos M. Combination of fibrin-agarose hydrogels and adipose-derived mesenchymal stem cells for peripheral nerve regeneration. J. Neural. Eng., 2013, 10:026022.

Carriel V, Garzón I, Alaminos M, Cornelissen M. Histological assessment in peripheral nerve tissue engineering. Neural. Regen. Res. 2014, 9:1657-60.

Carriel V, Garzón I, Campos A, Cornelissen M, Alaminos M. Differential expression of GAP-43 and neurofilament during peripheral nerve regeneration through bio-artificial conduits. J. Tissue Eng. Regen. Med. 2015, in press.

Challa SR. Surgical animal models of neuropathic pain: Pros and Cons. Int. J. Neurosci. 2014, in press.

Chen L, Qin J, Cheng C, Niu S, Liu Y, Shi S, Liu H, Shen A. Spatiotemporal expression of SSeCKS in injured rat sciatic nerve. Anat. Rec., 2008, 291:527-37.

Chen LE, Seaber AV, Glisson RR, Davies H, Murrell GA, Anthony DC, Urbaniak JR. The functional recovery of peripheral nerves following defined acute crush injuries. J. Orthop. Res., 1992, 10:657-64.

Chen XY, Yin YF, Zhang TT, Zhao YH, Yang YM, Yu XM, Wang HK. Ultrasound imaging of chitosan nerve conduits that bridge sciatic nerve defects in rats. Neural Regen. Res., 2014, 9:1386-1388.

Chiu DT, Strauch B. A prospective clinical evaluation of autogenous vein grafts used as a nerve conduit for distal sensory nerve defects of 3 cm or less. Plast. Reconstr. Surg., 1990, 86:928-34.

Chow R, Yan W, Armati P. Electrophysiological effects of single point transcutaneous 650 and 808 nm laser irradiation of rat sciatic nerve: a study of relevance for low-level laser therapy and laser acupuncture. Photomed. Laser Surg., 2012, 30:530-5.

Cirillo V, Clements BA, Guarino V, Bushman J, Kohn J, Ambrosio L. A comparison of the performance of mono- and bi-component electrospun conduits in a rat sciatic model. Biomaterials, 2014, 35:8970-82.

Cobianchi S, de Cruz J, Navarro X. Assessment of sensory thresholds and nociceptive fiber growth after sciatic nerve injury reveals the differential contribution of collateral reinnervation and nerve regeneration to neuropathic pain. Exp. Neurol., 2014, 255:1-11.

Costa LM, Simões MJ, Maurício AC, Varejão AS. Methods and protocols in peripheral nerve regeneration experimental research: part IV-kinematic gait analysis to quantify peripheral nerve regeneration in the rat. Int. Rev. Neurobiol., 2009, 87:127-39.

Dadon-Nachum M, Melamed E, Offen D. Stem cells treatment for sciatic nerve injury. Expert Opin. Biol. Ther., 2011, 11:1591-7.

De Leon M, Welcher AA, Suter U, Shooter EM. Identification of transcriptionally regulated genes after sciatic nerve injury. J. Neurosci Res., 1991, 29:437-48.

De Medinaceli L, Freed WJ, Wyatt RJ. An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks. Exp. Neurol., 1982, 77:634-43

De Paola MM, Buanne P, Biordi L, Bertini R, Ghezzi P, Mennini T. Chemokine MIP-2/CXCL2, acting on CXCR2, induces motor neuron death in primary cultures. Neuroimmunomodulation, 2007, 14:310-16.

Dellon AL, Mackinnon SE. Sciatic nerve regeneration in the rat. Validity of walking track assessment in the presence of chronic contractures. Microsurgery, 1989, 10:220-5.

Deumens R, Jaken RJ, Marcus MA, Joosten EA. The CatWalk gait analysis in assessment of both dynamic and static gait changes after adult rat sciatic nerve resection. J. Neurosci. Methods, 2007;164:120-30.

Dinh P, Hazel A, Palispis W, Suryadevara S, Gupta R. BBB Functional assessment after sciatic nerve injury in a rat model. Microsurgery, 2009, 29:644-9.

Di Scipio F, Raimondo S, Tos P, Geuna S. A simple protocol for paraffin-embedded myelin sheath staining with osmium tetroxide for light microscope observation. Microsc. Res. Tech., 2008, 71:497-502.

Dodla MC, Bellamkonda RV. Differences between the effect of anisotropic and isotropic laminin and nerve growth factor presenting scaffolds on nerve regeneration across long peripheral nerve gaps. Biomaterials, 2008, 29:33-46.

Eijkelkamp N, Heijnen CJ, Willemen HL, Deumens R, Joosten EA, Kleibeuker W, denHartog IJ, van Velthoven CT, Nijboer C, Nassar MA, Dorn GW 2nd, Wood JN,Kavelaars A. GRK2: a novel cell-specific regulator of severity and duration ofinflammatory pain. J. Neurosci., 2010a, 30:2138-49.

Evans GR. Peripheral nerve injury: a review and approach to tissue engineered constructs. Anat. Rec., 2001;263:396-404.

Félix SP, Pereira Lopes FR, Marques SA, Martinez AM. Comparison between suture and fibrin glue on repair by direct coaptation or tubulization of injured mouse sciatic nerve. Microsurgery, 2013, 33:468-77.

Fleming CE, Saraiva MJ, Sousa MM. Transthyretin enhances nerve regeneration. J. Neurochem., 2007, 103:831-9.

Fornaro M, Lee JM, Raimondo S, Nicolino S, Geuna S, Giacobini-Robecchi M. Neuronal intermediate filament expression in rat dorsal root ganglia sensory neurons: an in vivo and in vitro study. Neuroscience, 2008, 153:1153-63.

Galtrey CM, Fawcett JW. Characterization of tests of functional recovery after median and ulnar nerve injury and repair in the rat forelimb. J. Peripher. Nerv. Syst., 2007;12:11-27.

Gao H, You Y, Zhang G, Zhao F, Sha Z, Shen Y. The use of fiber-reinforced scaffolds cocultured with Schwann cells and vascular endothelial cells to repair rabbit sciatic nerve defect with vascularization. Biomed Res. Int., 2013, 2013:362918.

Geuna S, Raimondo S, Ronchi G, Di Scipio F, Tos P, Czaja K, Fornaro M. Histology of the peripheral nerve and changes occurring during nerve regeneration. Int. Rev. Neurobiol., 2009, 87:27-46.

Geuna S, Tos P, Battiston B, Giacobini-Robecchi MG. Bridging peripheral nerve defects with muscle-vein combined guides. Neurol. Res., 2004, 26:139-44

Geuna S, Tos P, Battiston B, Guglielmone R, Giacobini-Robecchi MG. Morphological analysis of peripheral nerve regenerated by means of vein grafts filled with fresh skeletal muscle. Anat. Embryol., 2000, 201:475-82.

Gigo-Benato D, Russo TL, Geuna S, Domingues NR, Salvini TF, Parizotto NA. Electrical stimulation impairs early functional recovery and accentuates skeletal muscle atrophy after sciatic nerve crush injury in rats. Muscle Nerve, 2010, 41:685-93.

Gingras M, Beaulieu MM, Gagnon V, Durham HD, Berthod F. In vitro study of axonal migration and myelination of motor neurons in a three-dimensional tissue-engineered model. Glia, 2008, 56:354-64.

Glasby MA, Gschmeissner SG, Hitchcock RJI, Huang CL, de Souza BA. A comparison of nerve regeneration through nerve and muscle grafts in rat sciatic nerve. Neuroorthopaedics, 1986, 2:21-8.

Gramsbergen A, IJkema-Paassen J, Meek MF. Sciatic nerve transection in the adult rat: abnormal EMG patterns during locomotion by aberrant innervation of hindleg muscles. Exp. Neurol., 2000, 161:183-93.

Griffin JW, Hogan MV, Chhabra AB, Deal DN. Peripheral nerve repair and reconstruction. J Bone Joint Surg Am., 2013, ,95:2144-51.

Gupta R, Channual JC. Spatiotemporal pattern of macrophage recruitment after chronic nerve compression injury. J. Neurotrauma, 2006, 23:216-26.

Haastert-Talini K, Geuna S, Dahlin LB, Meyer C, Stenberg L, Freier T, Heimann C, Barwig C, Pinto LF, Raimondo S, Gambarotta G, Samy SR, Sousa N, Salgado AJ, Ratzka A, Wrobel S, Grothe C. Chitosan tubes of varying degrees of acetylation for bridging peripheral nerve defects. Biomaterials, 2013, 34:9886-904.

Hara K, Yasuhara T, Maki M, Matsukawa N, Masuda T, Yu SJ, Ali M, Yu G, Xu L, Kim SU, Hess DC, Borlongan CV. Neural progenitor NT2N cell lines from teratocarcinoma for transplantation therapy in stroke. Prog. Neurobiol., 2008, 85:318-34.

Hiroi S, Tsukamoto Y, Sasaki F, Miki N, Taira E. Involvement of gicerin, a cell adhesion molecule, in development and regeneration of chick sciatic nerve. FEBS Lett, 2003, 554(3):311-4.

Hsu SH, Chan SH, Chiang CM, Chen CC, Jiang CF. Peripheral nerve regeneration using a microporous polylactic acid asymmetric conduit in a rabbit long-gap sciatic nerve transection model. Biomaterials, 2011, 32:3764-75.

Isaacs J. Major peripheral nerve injuries. Hand Clin., 2013, 29:371-82.

Johansson F, Dahlin LB. The multiple silicone tube device, "tubes within a tube," for multiplication in nerve reconstruction. Biomed Res. Int., 2014, 2014:689127.

Kaplan S, Geuna S, Ronchi G, Ulkay MB, von Bartheld CS. Calibration of the stereological estimation of the number of myelinated axons in the rat sciatic nerve: a multicenter study. J. Neurosci. Methods, 2010, 187:90-9

Karsidag S, Akcal A, Sahin S, Karsidag S, Kabukcuoglu F, Ugurlu K. Neurophysiological and morphological responses to treatment with acetyl-L-carnitine in a sciatic nerve injury model: preliminary data. J. Hand Surg. Eur. Vol., 2012, 37:529-36.

Kilic A, Ojo B, Rajfer RA, Konopka G, Hagg D, Jang E, Akelina Y, Mao JJ, Rosenwasser MP, Tang P. Effect of white adipose tissue flap and insulin-like growth factor-1 on nerve regeneration in rats. Microsurgery, 2013, 33:367-75.

Kingery WS, Lu JD, Roffers JA, Kell DR. The resolution of neuropathic hyperalgesia following motor and sensory functional recovery in sciatic axonotmetic mononeuropathies. Pain, 1994;58:157-68.

Kršljak E, Stajčić Z. Evidence that ampicillin and streptomycin can suppress autotomic behaviour in rats. Acta Veterinaria, 54:145-52.

Kuffler DP. Ultrasound imaging of regenerating rat sciatic nerves in situ. J. Neurosci. Methods, 2010, 188:276-9.

Larsen JO. Related Stereology of nerve cross sections. J Neurosci Methods 1998; 85: 107-18.

Liao CD, Zhang F, Guo RM, Zhong XM, Zhu J, Wen XH, Shen J. Peripheral nerve repair: monitoring by using gadofluorine M-enhanced MR imaging with chitosan nerve conduits with cultured mesenchymal stem cells in rat model of neurotmesis. Radiology, 2012, 262:161-71.

Long L, Huang Y, Wu H, Luan W, Zhang Q, Wen H, Ding T, Wang Y. Dynamic change of Prohibitin2 expression in rat sciatic nerve after crush. Cell. Mol. Neurobiol., 2013, 33:689-98

Lou D, Sun B, Wei H, Deng X, Chen H, Xu D, Li G, Xu H, Wang Y. Spatiotemporal expression of testicular protein kinase 1 after rat sciatic nerve injury. J. Mol. Neurosci., 2012, 47:180-91.

Lutz BS, Chuang DC, Hsu JC, Ma SF, Wei FC. Selection of donor nerves--an important factor in end-to-side neurorrhaphy. Br. J. Plast. Surg., 2000;53:149-54.

Manoli T, Schulz L, Stahl S, Jaminet P, Schaller HE. Evaluation of sensory recovery after reconstruction of digital nerves of the hand using muscle-in-vein conduits in comparison to nerve suture or nerve autografting. Microsurgery, 2014, in press.

Marcoccio I, Vigasio A. Muscle-in-vein nerve guide for secondary reconstruction in digital nerve lesions. J. Hand Surg. Am., 2010, 35:1418-26.

Masters DB, Berde CB, Dutta SK, Griggs CT, Hu D, Kupsky W, Langer R. Prolonged regional nerve blockade by controlled release of local anesthetic from a biodegradable polymer matrix. Anesthesiology, 1993, 79:340-6.

Moimas S, Novati F, Ronchi G, Zacchigna S, Fregnan F, Zentilin L, Papa G, Giacca M, Geuna S, Perroteau I, Arnež ZM, Raimondo S. Effect of vascular endothelial growth factor gene therapy on post-traumatic peripheral nerve regeneration and denervation-related muscle atrophy. Gene Ther., 2013, 20:1014-21.

Moreno-Flores MT, Bradbury EJ, Martín-Bermejo MJ, Agudo M, Lim F, Pastrana E, Avila J, Diaz-Nido J, McMahon SB, Wandosell F. A clonal cell line from immortalized olfactory

ensheathing glia promotes functional recovery in the injured spinal cord. Mol. Ther., 2006, 13:598-608.

Navarro X, Kennedy WR. Sweat gland reinnervation by sudomotor regeneration after different types of lesions and graft repairs. Exp. Neurol., 1989;104:229-34.

Navarro X, Udina E. Methods and protocols in peripheral nerve regeneration experimental research: part III-electrophysiological evaluation. Int. Rev. Neurobiol., 2009, 87:105-26.

Nichols CM, Myckatyn TM, Rickman SR, Fox IK, Hadlock T, Mackinnon SE. Choosing the correct functional assay: A comprehensive assessment of functional tests in the rat. Behav. Brain. Res., 2005;163:143-158.

Nijhuis TH, Bodar CW, van Neck JW, Walbeehm ET, Siemionow M, Madajka M, Cwykiel J, Blok JH, Hovius SE. Natural conduits for bridging a 15-mm nerve defect: comparison of the vein supported by muscle and bone marrow stromal cells with a nerve autograft. J. Plast. Reconstr. Aesthet. Surg., 2013, 66:251-9

Oliveira EF, Mazzer N, Barbieri CH, Selli M. Correlation between functional index and morphometry to evaluate recovery of the rat sciatic nerve following crush injury: experimental study. J. Reconstr. Microsurg., 2001;17:69-75.

Papalia I, Tos P, Stagno d'Alcontres F, Battiston B, Geuna S. On the use of the grasping test in the rat median nerve model: a re-appraisal of its efficacy for quantitative assessment of motor function recovery. J. Neurosci. Methods, 2003;127:43-7.

Pereira JE, Cabrita AM, Filipe VM, Bulas-Cruz J, Couto PA, Melo-Pinto P, Costa LM, Geuna S, Maurício AC, Varejão AS. A comparison analysis of hindlimb kinematics during overground and treadmill locomotion in rats. Behav. Brain Res., 2006, 172:212-8.

Pereira JH, Bowden REM, Gattuso JM, Norris RW. Comparison of results of repair of digital nerves by denatured muscle grafts and end-to-end sutures. *J. Hand. Surg., 1991;* 16**:** 519-23.

Que J, Cao Q, Sui T, Du S, Kong D, Cao X. Effect of FK506 in reducing scar formation by inducing fibroblast apoptosis after sciatic nerve injury in rats. Cell Death Dis., 2013, 4:e526.

Radevik B, Lundborg G. Permeability of intraneural microvessels and perineurium following acute, graded experimental nerve compression. Scand. J. Plast. Reconstr. Surg., 1977;11:179-87.

Raimondo S, Fornaro M, Di Scipio F, Ronchi G, Giacobini-Robecchi MG, Geuna S. Methods and protocols in peripheral nerve regeneration experimental research: part II-morphological techniques. Int. Rev. Neurobiol., 2009, 87:81-103.

Rao P, Kotwal PP, Farooque M, Dinda AK. Muscle autografts in nerve gaps. Pattern of regeneration and myelination in various lengths of graft: an experimental study in guinea pigs. J. Orthop. Sci., 2001, 6:527-34.

Reid AJ, de Luca AC, Faroni A, Downes S, Sun M, Terenghi G, Kingham PJ. Long term peripheral nerve regeneration using a novel PCL nerve conduit. Neurosci. Lett., 2013, 544:125-30.

Rodríguez FJ, Verdú E, Ceballos D, Navarro X. Nerve guides seeded with autologous schwann cells improve nerve regeneration. Exp. Neurol., 2000, 161:571-84.

Ronchi G, Raimondo S, Varejão AS, Tos P, Perroteau I, Geuna S. Standardized crush injury of the mouse median nerve. J. Neurosci. Methods, 2010, 188:71-5.

Rosberg HE, Carlsson KS, Cederlund RI, Ramel E, Dahlin LB. Costs and outcome for serious hand and arm injuries during the first year after trauma - a prospective study. BMC Public Health, 2013,13:501.

Rupp A, Dornseifer U, Fischer A, Schmahl W, Rodenacker K, Jütting U, Gais P, Biemer E, Papadopulos N, Matiasek K. Electrophysiologic assessment of sciatic nerve regeneration in the rat: surrounding limb muscles feature strongly in recordings from the gastrocnemius muscle. J. Neurosci. Methods, 2007, 166:266-77.

Rupp A, Schmahl W, Lederer W, Matiasek K. Strain differences in the branching of the sciatic nerve in rats. Anat. Histol. Embryol., 2007b, 36:202-8.

Russell WM, Burch RL. The Principles of Humane Experimental Technique. Universities Federation for Animal Welfare (UFAW) Blackwell Publishing: Hertfordshire, 1992.

Russo TL, Peviani SM, Freria CM, Gigo-Benato D, Geuna S, Salvini TF. Electrical stimulation based on chronaxie reduces atrogin-1 and myoD gene expressions in denervated rat muscle. Muscle Nerve, 2007, 35:87-97.

Saito H, Kanje M, Dahlin LB. Delayed nerve repair increases number of caspase 3 stained Schwann cells. Neurosci. Lett., 2009, 456:30-3.

Sak K, Illes P. Neuronal and glial cell lines as model systems for studying P2Y receptor pharmacology. Neurochem. Int., 2005 47:401-12.

Sarikcioglu L, Yaba A, Tanriover G, Demirtop A, Demir N, Ozkan O. Effect of severe crush injury on axonal regeneration: a functional and ultrastructural study. J. Reconstr. Microsurg., 2006;23:143-9.

Savastano LE, Laurito SR, Fitt MR, Rasmussen JA, Gonzalez Polo V, Patterson SI. Sciatic nerve injury: a simple and subtle model for investigating many aspects of nervous system damage and recovery. J Neurosci Methods., 2014, 227:166-80.

Scanlin HL, Carroll EA, Jenkins VK, Balkowiec A. Endomorphin-2 is released from newborn rat primary sensory neurons in a frequency- and calcium-dependent manner. Eur. J. Neurosci., 2008, 27:2629-42.

Schmalbruch H. Fiber composition of the rat sciatic nerve. Anat. Rec., 1986, 215:71-81

Shastry P., Basu A, Rajadhyaksha MS. Neuroblastoma cell lines--a versatile in vitro model in neurobiology. Int. J. Neurosci., 2001, 108:109-26.

Siddique R, Vyas A, Thakor N, Brushart TM. A two-compartment organotypic model of mammalian peripheral nerve repair. J. Neurosci. Methods, 2014, 232:84-92.

Siemionow M, Brzezicki G. Current techniques and concepts in peripheral nerve repair. Int. Rev. Neurobiol., 2009, 87:141-72.

Siironen J, Vuorio E, Sandberg M, Röyttä M. Expression of type I and III collagen and laminin beta1 after rat sciatic nerve crush injury. J. Peripher. Nerv. Syst., 1996, 1:209-21.

Sinis N, Schaller HE, Becker ST, Lanaras T, Schulte-Eversum C, Muller HW, Vonthein R, Rosner H, Haerle M. Cross-chest median nerve transfer: a new model for the evaluation of nerve regeneration across a 40 mm gap in the rat. J. Neurosci. Methods, 2006;156:166-72.

Sinis N, Di Scipio F, Schönle P, Werdin F, Kraus A, Koopmanns G, Masanneck C, Hermanns S, Danker T, Guenther E, Haerle M, Schaller HE, Geuna S, Mueller HW. Local administration of

DFO-loaded lipid particles improves recovery after end-to-end reconstruction of rat median nerve. Restor. Neurol. Neurosci., 2009, 27:651-62.

Sta M, Cappaert NL, Ramekers D, Baas F, Wadman WJ. The functional and morphological characteristics of sciatic nerve degeneration and regeneration after crush injury in rats. J. Neurosci. Methods., 2014,222:189-98.

Sufan W, Suzuki Y, Tanihara M, Ohnishi K, Suzuki K, Endo K, Nishimura Y. Sciatic nerve regeneration through alginate with tubulation or nontubulation repair in cat. J. Neurotrauma, 2001, 18:329-38.

Tos P, Battiston B, Ciclamini D, Geuna S, Artiaco S. Primary repair of crush nerve injuries by means of biological tubulization with muscle-vein-combined grafts. Microsurgery, 2012, 32:358-63.

Tos P, Ronchi G, Nicolino S, Audisio C, Raimondo S, Fornaro M, Battiston B, Graziani A, Perroteau I, Geuna S. Employment of the mouse median nerve model for the experimental assessment of peripheral nerve regeneration. J. Neurosci. Methods, 2008, 169:119-27.

Tos P, Ronchi G, Papalia I, Sallen V, Legagneux J, Geuna S, Giacobini-Robecchi MG. Methods and protocols in peripheral nerve regeneration experimental research: part I-experimental models. Int. Rev Neurobiol., 2009, 87:47-79.

Tos P, Ronchi. G, Geuna S, Battiston B. Future perspectives in nerve repair and regeneration. Int. Rev. Neurobiol., 2013, 109:165-92.

Toth C, Martinez JA, Liu WQ, Diggle J, Guo GF, Ramji N, Mi R, Hoke A, Zochodne DW. Local erythropoietin signaling enhances regeneration in peripheral axons. Neuroscience, 2008, 154:767-83.

Trotter J. The development of myelin-forming glia: studies with primary cell cultures and immortalized cell lines. Perspect. Dev. Neurobiol., 1993, 1:149-54.

Uranüs S, Bretthauer G, Nagele-Moser D, Saliba S, Tomasch G, Rafolt D, Justich I, Waldert J, Berghold A, Kleinert R, Becker H, Voges U, Wiederstein-Grasser I, Koch H. New synthetic

prosthesis for peripheral nerve injuries: an experimental pilot study. Surg. Innov., 2013, 20:171-5.

Varejão AS, Cabrita AM, Geuna S, Patrício JA, Azevedo HR, Ferreira AJ, Meek MF. Functional assessment of sciatic nerve recovery: biodegradable poly (DLLA-epsilon-CL) nerve guide filled with fresh skeletal muscle. Microsurgery, 2003, 23:346-53.

Varejão AS, Meek MF, Ferreira AJ, Patrício JA, Cabrita AM. Functional evaluation of peripheral nerve regeneration in the rat: walking track analysis. J. Neurosci. Methods., 2001, 15;108:1-9

Varejão AS, Melo-Pinto P, Meek MF, Filipe VM, Bulas-Cruz J. Methods for the experimental functional assessment of rat sciatic nerve regeneration. Neurol. Res., 2004, 26:186-94.

Varejão ASP, Cabrita AM, Meek MF, Bulas-Cruz J, Melo-Pinto P, Raimondo S, Geuna S, Giacobini-Robecchi MG. Functional and morphological assessment of a standardized rat sciatic nerve crush injury with a non-serrated clamp. J. Neurotrauma, 2004;21:1652-70.

Wang CZ, Chen YJ, Wang YH, Yeh ML, Huang MH, Ho ML, Liang JI, Chen CH. Low-level laser irradiation improves functional recovery and nerve regeneration in sciatic nerve crush rat injury model. PLoS One. 2014 Aug 13;9(8):e103348.

Wang H, Spinner RJ, Sorenson EJ, Windebank AJ. Measurement of forelimb function by digital video motion analysis in rat nerve transection models. J. Peripher. Nerv. Syst., 2008;13:92-102.

Whishaw IQ, Pellis SM, Gorny BP. Skilled reaching in rats and humans: evidence for parallel development or homology. Behav. Brain Res., 1992;47:59-70.

Whitlock EL, Tuffaha SH, Luciano JP, Yan Y, Hunter DA, Magill CK, Moore AM, Tong AY, Mackinnon SE, Borschel GH. Processed allografts and type I collagen conduits for repair of peripheral nerve gaps. Muscle Nerve, 2009, 39:787-99.

Willemen HL, Eijkelkamp N, Wang H, Dantzer R, Dorn GW 2nd, Kelley KW, HeijnenCJ, Kavelaars A. Microglial/macrophage GRK2 determines duration of peripheralIL-1beta-induced hyperalgesia: contribution of spinal cord CX3CR1, p38 and IL-1signaling. Pain, 2010, 150:550-60.

Wright MC, Mi R, Connor E, Reed N, Vyas A, Alspalter M, Coppola G, Geschwind DH, Brushart TM, Höke A. Novel roles for osteopontin and clusterin in peripheral motor and sensory axon regeneration. J. Neurosci., 2014, 34:1689-700.

Xue C, Hu N, Gu Y, Yang Y, Liu Y, Liu J, Ding F, Gu X. Joint use of a chitosan/PLGA scaffold and MSCs to bridge an extra large gap in dog sciatic nerve. Neurorehabil. Neural Repair, 2012, 26:96-106.

Yamasaki T, Fujiwara H, Oda R, Mikami Y, Ikeda T, Nagae M, Shirai T, Morisaki S, Ikoma K, Masugi-Tokita M, Yamada K, Kawata M, Kubo T. In vivo evaluation of rabbit sciatic nerve regeneration with diffusion tensor imaging (DTI): correlations with histology and behavior. Magn. Reson. Imaging, 2015, 33:95-101.