RESEARCH ARTICLE

Animal models used to study direct peripheral nerve repair: a systematic review

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Abstract

Objective: Peripheral nerve repair is required after traumatic injury. This common condition represents a major public health problem worldwide. Recovery after nerve repair depends on several factors, including the severity of the injury, the nerve involved, and the surgeon's technical skills. Despite the precise microsurgical repair of nerve lesions, adequate functional recovery is not always achieved and, therefore, the regeneration process and surgical techniques are still being studied. Pre-clinical animal models are essential for this research and, for this reason, the focus of the present systematic review (according to the PRISMA statement) was to analyze the different animal models used in pre-clinical peripheral nerve repair studies.
Data sources: Original articles, published in English from 2000 to 2018, were collected using the Web of Science, Scopus, and PubMed databases.
Data selection: Only preclinical trials on direct nerve repair were included in this review. The articles were evaluated by the first two authors, in accordance with predefined data fields.
Outcome measures: The primary outcomes included functional motor abilities, daily activity and regeneration rate. Secondary outcomes included coaptation technique and animal model.
Results: This review yielded 267 articles, of which, after completion of the screening, 49 studies were analyzed. There were 1425 animals in those 49 studies, being rats, mice, guinea pigs, rabbits, cats and dogs the different pre-clinical models. The nerves used were classified into three groups: head and neck (11), forelimb (8) and hindlimb (30). The techniques used to perform the coaptation were: microsuture (46), glue (12), laser (8) and mechanical (2). The follow-up examinations were histology (43), electrophysiological analysis (24) and behavioral observation (22).
Conclusion: The most widely used animal model in the study of peripheral nerve repair is the rat. Other animal models are also used but the cost-benefit of the rat model provides several strengths over the others. Suture techniques are currently the first option for nerve repair, but the use of glues, lasers and bioengineering materials is increasing. Hence, further research in this field is required to improve clinical practice.

Key Words: *nerve; microsurgery; peripheral nerve; regeneration; repair; reconstruction; direct nerve repair; animal model; coaptation; PRISMA; systematic review*

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Introduction

Peripheral nerve injury is a common disorder in society, with approximately 1 million patients requiring peripheral nerve surgery worldwide every year (Daly et al., 2012). There are many causes of nerve injury: crush, ischemia, sharp damage, traction, and, lesin this field s commonly, electric shock and vibration (Robinson, 2000, 2004). Road accidents are the primary cause of nerve trauma in the civilian population (Huckhagel et al., 2018a) while gunshots wounds, bombs and other explosive devices are the most common causes of nerve trauma in military conditions (Birch et al., 2012). Indeed, the first attempts at nerve repair were initiated by military surgeons during and after wars.

One-third of peripheral nerve injuries are a result of lacerations by sharp objects or long bone fractures (Siemionow and Brzezicki, 2009), with almost three quarters of all nerve injuries occurring in the upper limbs (Huckhagel et al., 2018b), especially affecting the ulnar nerve (Kouyoumdjian, 2006). Although axon regeneration has been studied for more than a century, it is still proving a challenge to obtain good functional results regarding nerve repair. There are many factors affecting nerve repair following reconstruction, such as time between injury and treatment, patient age, severity of injury, extension and type of injury (Dvali and Mackinnon, 2007). Furthermore, the technical skills and strategies used by physicians can also affect the success of regeneration. The introduction of microsurgical techniques for nerve repair in 1964 (Smith, 1964) improved the outcomes of nerve reconstruction. The first approach to repair an injured nerve is the end-to-end (ETE) coaptation, performed using epineurial or perineurial suture techniques. However, the types of nerve repair procedures have been extended with different techniques such as end-to-side (ETS) repairs and nerve transfers. One of the most important aspects that should be taken into account in nerve repair is tension, with tensionless repairs shown to result in better outcomes (Griffin et al., 2014).

Understanding nerve regeneration and physiology is crucial to improve functional recovery after peripheral nerve

damage and, therefore, further research is needed. Firstly, *in vitro* studies are required to assess the toxicity and biocompatibility of different drugs, products and materials, while reducing the use of experimental animals according to the Three Rs (3Rs) principle (Kilkenny et al., 2010). However, these assays need to be followed by *in vivo* studies to investigate tissue reaction, immune system response, vascularization, mechanical function and other variables (Angius et al., 2012). Experimental animals have long been used for research and the results obtained have undoubtedly improved the quality and efficacy of medicine and health. Rodents, carnivores, lagomorphs, pigs, small ruminants and apes are the most common animals used in neuroscience (Mohanty et al., 2019). An ideal translational animal model must reproduce the specific processes that occur in human peripheral nerve injuries. However, each animal model has its own drawbacks and advantages. The identification of appropriate animal models, and their limitations and benefits, is required to produce pre-clinical scientific evidence prior to the development of human clinical trials (Sanders and Young, 1942).

The quality of reporting in systematic reviews (SRs) is not optimal, and only about 10% of SRs report working from a protocol (Moher et al., 2009). Moreover, SRs may fail if the authors do not report the risk of bias in the included studies– an assessment critical to the SR process. The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement is a 27 item checklist (Moher et al., 2009) that helps researchers improve their SR. With this checklist, the transparency and accountability of SRs will be improved. Simultaneously, if the protocols are registered it will be possible to reduce the number of reviews addressing the same question.

The main objective of this systematic review was to evaluate the different preclinical studies on direct peripheral nerve repair developed between 2000 and 2018, to assess the advantages and disadvantages of each animal model. As specific objectives, we want to study which animal model is better for the different nerves, what examinations we can perform to check the nerve recovery progress and the different techniques that surgeons can use in order to repair nerve injuries.

Data and Methods

Protocol and registration

This SR was performed according to the PRISMA statement (Moher et al., 2009). Being a SR, approval from an ethical committee was not necessary. The search strategy was performed by the first two authors, using the Web of Science, Scopus, and PubMed databases. This review protocol has no PROSPERO registration number because the outcomes are not directly related to human health, thus it is not eligible for inclusion in the International Prospective Register of Systematic Reviews.

Eligibility criteria

This SR of animal studies was not possible to apply all the PICOS (Population, Interventions, Comparator, outcomes and Study design), required on PRISMA statement, since PRISMA was originally devised for clinical trials.

The types of participants considered for this SR were animal models used in peripheral nerve studies, such as mouse, rat, guinea pig, rabbit, cat and dog.

The type of intervention referred to the different preclinical trials used in order to evaluate the best animal model depending on the technique used (ETE or ETS neurorrhaphy) for the peripheral nerve repair and regeneration.

The type of outcomes were measured in respect to nerve repair and regeneration, good level of muscle reinnervation, improvement of axon fibers number, better quality of the regeneration process, the regenerative processes of peripheral nerves after intervention, and the behavioral evolution of animals.

The types of studies eligible for this review were original preclinical trials evaluating direct peripheral nerve repair techniques. Reviews and meeting abstracts were not included. Studies concerning spinal cord or roots, cadaver, robot, flaps, grafts, cranial nerves, training, teaching and transplantation were also excluded.

Information source and search

Studies were identified by searching three electronic databases: Web of Science, Scopus, and PubMed. These databases were systematically searched for English language papers (published from January 2000 to December 2018) by entering the following keywords and Boolean operators: TS=(nerve* AND microsurg* AND (anastomos* OR direct repair) AND (rat OR mouse OR rabbit OR cat OR guinea pig OR dog OR animal)) in Web of Science; and nerve* AND microsurg* AND (anastomos* OR direct repair) AND (rat OR mouse OR rabbit OR cat OR guinea pig OR dog OR animal) in Scopus and PubMed.

Study selection

The titles and abstracts were evaluated for inclusion or exclusion and, when one could not be discarded, the full text of the article was acquired. The flow diagram (**Figure 1**) details the progression of studies that were collected or excluded, with reasons, in this SR. After reading all of the papers, the authors discussed them and resolved any disagreements by consensus.

Data collection process

The data collected from the papers was recorded in a table (**Table 1**) for later analysis. The information extracted from articles was: First author, year of publication, title, and journal (in order to organize them); technique used to perform the neurorrhaphies (suture, glue, laser and mechanical); animal model (number of animals used, groups of study, species, nerve model, anesthesia used and the duration of the study); and follow-up exams (histology, electrophysiological study and behavioral observations). Also, a brief conclusion and comments on each article were noted (data not shown).

Risk of bias in individual studies

In order to ascertain the validity of the eligible studies, the

Table 1 Summary of the studies included in this review about peripheral nerve repair

Study	Material	Species	Technique	Nerve	Anesthesia	Time	Measures
Adel et al. (2017)	10-0 polyamide, fibrin glue, epineurial	Rat Sprague-Dawley	ETE	Sciatic	IP	4 weeks	Pathological changes; epineurial thickness; structure and cross-sectional nerve diameter.
Al-Qattan (2000)	10-0 polypropylene, epineurial	Rat Sprague-Dawley	ETS	Sciatic	IM	12 weeks	Pathological changes.
Atta et al. (2012)	8-0 nylon, fibrin glue, epineurial	Dog	ETE	Facial	IM	16 weeks	Nerve conduction velocity; axon fibers count.
Bao et al. (2016)	10-0, epineurial	Rat Sprague-Dawley	ETE	MCN and Median	IP	8 and 12 weeks	Grooming test; axon fiber count; CMAP.
Beer et al. (2004)	10-0 nylon, epineurial	Rabbit New Zealand	ETE	Peroneal	INH	15 weeks	Nerve conduction velocity; CAPs; toe spreading reflex; axon number and diameter; histomorphometry; muscle weight.
Bhatt et al. (2017a)	9-0 nylon, KTP laser, epineurial	Rat Sprague-Dawley	ETE	Tibial	IP	6 weeks	Axon fibers count; walking track analysis.
Bhatt et al. (2017b)	9-0 nylon, epineurial CO_2 KTP lasers	Rat Sprague-Dawley	ETE	Tibial	IP	6 weeks	Walking track analysis; force threshold analysis.
Cho et al. (2010)	10-0 nylon, perineurial	Guinea pig	ETE	Facial	IP	6 weeks	Vibrissae and eye closure; electromyography; MAPs; myelinated axon fibers count.
Choi et al. (2004)	10-0 nylon, epineurial, cyanoacrylate	Rat Sprague-Dawley	ETE	Sciatic	IP	12 weeks	Axon fibers count; neurotization.
Dourado et al. (2004)	10-0 nylon, fibrin glue, epineurial	Rabbit New Zealand	ETE	Facial	SC	2, 4, 8 and 16 weeks	Nerve conduction velocity; axon fibers count.
Fekrazad et al. (2017)	10-0 polypropylene, diode laser	Rat Wistar	ETE	Sciatic	ND	12 weeks	Inflammation; electromyography; CMAP; walking track analysis; foot print test.
Félix et al. (2013)	10-0 nylon, fibrin glue, epineurial	Mouse C5/B16	ETE	Sciatic	IP	8 weeks	Foot print test; sciatic functional index; axon fibers count.
Fox et al. (2012)	9-0 nylon, epineurial	Rat Lewis	ETE	Sciatic	IM	4, 12, 24, 36, 48 and 96 weeks	Wallerian degeneration; axon fibers count.
Giovanoli et al. (2000)	11-0 nylon, epineurial	Rabbit New Zealand	ETS	Femoral	SC	32 weeks	Axon fibers count; muscle weight; muscle force evaluation.
Hasturk et al. (2018)	8-0 polypropylene, epineurial	Rat Wistar	ETE	Sciatic	IP	12 weeks	Myelin thickness; axon fibers count.
Howard et al. (2000)	10-0 nylon, epineurial	Rat Sprague-Dawley	ETE	Sciatic and Tibial	ND	12 weeks	Walking track analysis; force threshold analysis; foot print.
Hu et al. (2009)	10-0 nylon, epineurial	Cat	ETE	Vagus-Hypoglossal	IP	45 weeks	Wallerian degeneration; horseradish peroxidase tracing; histochemistry; tissue processing.
Hwang et al. (2005)	9-0 nylon, CO_2 laser, epineurial	Rat Sprague-Dawley	ETE	Facial	IP	6 weeks	Axon fibers count.
Hwang et al. (2006)	9-0 nylon, CO_2 laser, epineurial	Rat Sprague-Dawley	ETE	Hypoglossal-Facial	IP	4 and 8 weeks	Axon fibers count.
Hwang et al. (2008)	9-0 nylon, CO_2 laser, epineurial	Rabbit New Zealand	ETE	Hypoglossal-Facial	IM	6 weeks	Axon fibers count.
Isaacs et al. (2005)	10-0 nylon, epineurial.	Rat Sprague-Dawley	ETS	Tibial and Peroneal	IP	12 weeks	Muscle contraction force; axon fibers count.
Isla et al. (2003)	10-0 nylon, full thickness	Rat Wistar	ETE	Ulnar	SC	12 weeks	Electromyography; gait analysis; finger pinch; autophagy; pathological changes.
Knox et al. (2013)	10-0 nylon, fibrin glue, epineurial	Rat Wistar Hannover	ETE	Facial	IP	15 weeks	Whisking recovery.
Kokkalis et al. (2009)	11-0 nylon, perineurial	Rat Sprague-Dawley	ETS / ETE	MCN and Median	INH	4 weeks	Terzis grooming test; electromyography; muscle weight; axon fibers count; myelin thickness.
Kostopoulos et al. (2009)	11-0 nylon, perineurial	Rat Sprague-Dawley	ETS	MCN and Median	IP	1, 2, 3 and 4 weeks	Muscle weight. Terzis grooming test; axon fibers count; myelin thickness; CAPs.
Landegren et al. (2006)	9-0 nylon, epineurial, cyanoacrylate	Rat Sprague-Dawley	ETE	Sciatic	IP	24 weeks	Diameter myelinated axons; fiber density and number of myelinated axons; nerve action potential; nerve conduction velocity.
Liu et al. (2005)	Epineurial	Rat Sprague-Dawley	ETS / ETE	Recurrent Laryngeal	IP	12 weeks	Fiber optic laryngoscopy; electromyography.

Table 1 Continued

Study	Material	Species	Technique	Nerve	Anesthesia	Time	Measures
Liu et al. (2018)	10-0, epineurial	Rat Wistar	ETS/ETE	Facial–Hypoglossal	IP	16 and 32 weeks	Axon number, diameter and thickness; CMAP; vibrissae motor performance.
Lutz et al. (2000)	10-0 nylon, epineurial	Rat Sprague-Dawley	ETS/ETE	MCN	IP	4, 6, 8, 24 weeks	Wallerian degeneration; number of nerve fibers; grooming/grasping test; muscle contraction force.
Lutz and Lidman (2005)	10-0 nylon, 1.5 mm coupler, epineurial	Rat Sprague-Dawley	ETE	Sciatic	INH	22 weeks	Pinch reflex test; muscle contraction force; pathological changes.
Menovsky and Beek (2001)	10-0 PGA, CO_2 laser epineurial/perineurial	Rat Wistar	ETE	Sciatic	IP	1 and 6 weeks	Myelinated nerve fiber diameter; pathological changes; toe spreading test.
Menovsky and Beek (2003)	10-0 nylon, PGA or SS, CO_2 laser epineurial	Rat Wistar	ETE	Sciatic	IP	16 weeks	Pathological changes.
Nunes e Silva et al. (2010)	Fibrin glue, epineurial	Rat Wistar	ETS	Fibular	IP	12 weeks	Walking track analysis; diameter of the myelinated axons.
Nunes e Silva et al. (2012)	10-0 polyamide, fibrin glue, epineurial	Rat Wistar	ETS	Fibular	IP	12 weeks	Walking track analysis; diameter of the myelinated axons.
Omori et al. (2012)	10-0 nylon, epineurial	Rat Wistar	ETE	Sciatic and Saphenous	IP	12 weeks	Dry muscle weight ratios; neuromuscular junction; pathological changes.
Ozkan et al. (2005)	10-0, epineurial	Rat Wistar	ETE	Sciatic	IM	24 weeks	Muscle weight; walking track analysis; pinching test; histomorphometry; limb circumference and toe contracture index; pathological changes.
Papakonstantinou, K.C. et al. 2002	10-0 nylon, epineurial	Rat Sprague-Dawley	ETE	Sciatic and Saphenous	IP	7 weeks	Gait analysis; nerve conduction velocity and CAP; axon fibers count; sciatic functional index.
Papalia et al. (2012)	10-0, epineurial	Rat Wistar	ETS / ETE	Median and Ulnar	IP	40 weeks	Grasping test; muscle mass; fiber density; axon fiber diameter; myelin thickness.
Park et al. (2002)	9-0 nylon, Titanium clips, epineurial	Rabbit New Zealand	ETE	Sciatic	IV	4, 8 and 12 weeks	Wallerian degeneration; gross examination; axon number, diameter and thickness; electromyography.
Peker et al. (2005)	10-0, perineurial	Rat Sprague-Darley	ETE	Sciatic	IP	2, 4, 8, 12, 20 and 28 weeks	Myelin thickness; pathological changes; walking track analysis; print length; toe spread; intermediary toe spread.
Shamir et al. (2001)	10-0 nylon, epineurial	Rat Wistar	ETE	Sciatic	IP	10 weeks	Number of axon and diameter; somatosensory evoked potentials
Suri et al. (2002)	10-0 nylon, fibrin glue, epineurial	Rat Wistar	ETE	Sciatic	IP	1.5, 3, 4, 8 and 12 weeks	Axon thickness; assessment of myelin; pathological changes.
Tiangco et al. (2001)	11-0 nylon, epineurial	Rat Sprague-Dawley	ETS	MCN	IP	4 weeks	Myelin thickness/axon diameter ratios; Terzis grooming test.
Tos et al. (2008)	12-0 nylon, epineurial	Mouse FVB	ETE	Median	IM	11 weeks	Grasping test; axon number, diameter and thickness.
Wang et al. (2009)	10-0	Rat Sprague-Dawley	ETE	Facial	IP	1, 2, 4, 10 weeks	Immunochemistry; amplitude and nerve conduction; electromyography.
Wieken et al. (2003)	Fibrin glue, cyanoacrylate	Rat Sprague-Dawley	ETE	Sciatic	IP	1, 2, 3 and 4 weeks	Axon diameter; pathological changes.
Wu et al. (2013)	9-0 nylon, epineurial	Rat Lewis	ETE	Sciatic	ND	1, 4, 6, 8 and 12 weeks	Nerve conduction velocity (latency and amplitude of CMAP); myelinated nerve fibers and regeneration.
Yan, et al. (2002)	10-0 nylon, epineurial	Rat Sprague-Dawley	ETE	Peroneal	IP	14 weeks	Electromyography; tetanic force and moist weight of extensor digitorium longus muscles; axon fibers count.
Zhang et al. (2000)	10- 0 nylon, perineurial	Rat Sprague-Dawley	ETS	Peroneal	IP	32, 48 weeks	Dry muscular weight; nerve conduction velocity; myelinated axons.

In this table we describe: the type of suture (size, material, type of coaptation), laser or glue used, animal model, type of neurorrhaphy, nerve used for the procedure, anesthesia administration, time point and measurements (histology, electrophysiology and behavioral observations). CAP: Compound action potential; CMAP: compound muscle action potential; ETE: end-to-end; ETS: end-to-side; IM: intramuscular; INH: inhalational; IP: intraperitoneal; IV: intravenous; KTP: potassium titanyl phosphate; MAP: muscle action potential; MCN: musculocutaneous nerve; ND: not determined; PGA: polyglycolic acid; SC: subcutaneous; SS: stainless steel.

first two authors of this SR used the Systematic Review Centre for Laboratory Animal Experimentation's (SYRCLE's) risk of bias tool (Hooijmans et al., 2014) for animal studies. Ten entries related to 6 types of bias are contained in this risk of bias tool, and the types of bias were: selection, performance, detection, attrition, reporting and other bias. For each entry there was a signaling question that had to be answered with: "yes" (low risk of bias), "no" (high risk of bias) or "unclear" (unclear risk of bias). If one of those questions was answered with "no", indicates a high risk of bias for that entry.

The reviewers worked independently to determine the adequacy of randomization and concealment of allocation, baseline characteristics, blinding of patients and outcomes, randomization of housing and outcomes, incomplete data, and the selective reporting. The qualification of each risk of bias was categorized as low, high or unclear.

Statistical analysis

No statistical analyses were performed due to the big variety of species included in this study.

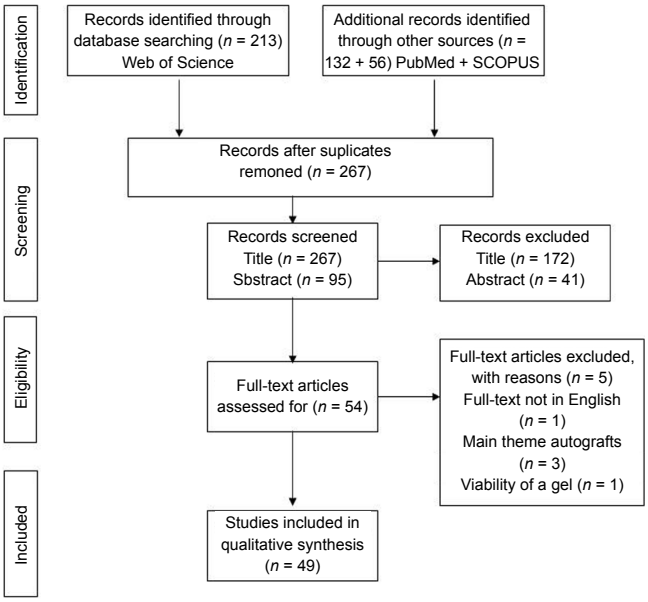

Figure 1 Flow diagram of study selection.

Results

Study selection

As depicted in the PRISMA flow diagram (**Figure 1**), we collected a total of 401 results from our search in the Web of Science, Scopus, and PubMed databases. After removing duplicates (134), 267 papers remained, which were recorded and screened according to their titles and abstracts. From these 267 articles, we excluded 172 because they were book chapters or reviews, or because their title did not fit with the criteria, then a further 41 that were judged not to accord with the criteria based on their abstracts. The full text of the remaining 54 articles was assessed for eligibility, of which one contained only the abstract in English, with the rest of the paper written in another language, so this paper was discarded. Finally, examination of the full text from the remaining 53 studies identified 3 articles about graft repair and 1 studying the viability of a gel, rather than nerve repair or regeneration. Once the exclusion of articles was complete, we had 49 studies that used small animal models: dog (1), cat (1), guinea pig (1), mouse (2), rabbit (5) and rat (39), and all of these were included in the qualitative synthesis.

Ultimately, 49 original articles about direct nerve repair and published in English were selected for inclusion in this review.

Study characteristics

Methods

First we analyzed the year in which each study was published, in order to assess the activity of research in this field. We classified the articles into groups of five year periods, and the results show a decrease in publication rate. In the first five years (2000–2004), 18 articles (Al-Qattan, 2000; Giovanoli et al., 2000; Howard et al., 2000; Lutz et al., 2000; Zhang et al., 2000; Menovsky and Beek, 2001, 2003; Shamir et al., 2001; Tiangco et al., 2001; Park et al., 2002; Suri et al., 2002; Yan et al., 2002; Isla et al., 2003; Wieken et al., 2003; Beer et al., 2004; Choi et al., 2004; Dourado et al., 2004; Cho et al., 2010) were recorded, which fell to 14 (Hwang et al., 2005, 2006, 2008; Isaacs et al., 2005; Liu et al., 2005; Lutz and Lidman, 2005; Ozkan et al., 2005; Peker et al., 2005; Landegren et al., 2006; Tos et al., 2008; Hu et al., 2009; Kokkalis et al., 2009; Kostopoulos et al., 2009; Wang et al., 2009) between 2005 and 2009, 10 (Cho et al., 2010; Nunes e Silva et al., 2010, 2012; Attar et al., 2012; Fox et al., 2012; Omori et al., 2012; Papalia et al., 2012; Félix et al., 2013; Knox et al., 2013; Wu et al., 2013) from 2010–2014, and finally 7 (Bao et al., 2016; Adel et al., 2017; Bhatt et al., 2017a, b; Fekrazad et al., 2017; Hasturk et al., 2018; Liu et al., 2018) published in the most recent period (2015–2018).

Animals

Of the 49 studies, 3 (Howard et al., 2000; Isla et al., 2003; Fox et al., 2012) contained some groups examining graft or gap repair, but those groups were not included in this SR.

From all of the studies included, a total of 1425 animals were used: 1226 rats, 101 rabbits, 27 mice, 39 cats, 24 guinea pigs, and 8 dogs.

Interventions

Not all of the studies employed the same nerve model, so we divided the articles into 3 groups: head and neck, forelimb, and hindlimb nerves. The head and neck group comprised 11 studies, using guinea pig (1), cat (1), dog (1), rabbit (2), and rat (6) animal models for the study of facial (6), hypoglossal (3), vagus (1), and recurrent laryngeal (1) nerves. For the forelimb group, we only found 8 studies, with mouse (1) and rat (7) used as animal models to focus on the musculocutaneous (5), median (5), and ulnar (2) nerves. In the hindlimb group, 30 studies were found, using mouse (1), rabbit (3), and rat (26); with the nerves of interest being the sciatic (21), peroneal (6), tibial (4), saphenous (1), and femoral (1). All of this information is documented in **Figure 2**.

There was high degree of variability in the duration of the studies, ranging from 1 to 96 weeks. Many studies (Lutz et al., 2000; Zhang et al., 2000; Papakonstantinou et al., 2002; Park et al., 2002; Suri et al., 2002; Menovsky and Beek, 2003; Wieken et al., 2003; Dourado et al., 2004; Hwang et al., 2005; Peker et al., 2005; Kostopoulos et al., 2009; Wang et al., 2009; Fox et al., 2012; Wu et al., 2013; Bao et al., 2016; Liu et al., 2018) have more than one end study period, which means that there are in total 89 end time periods out of the 49 selected studies. Similarly to the year of publication analysis, the length of the studies were grouped according to the following periods: 1 to 4 weeks (group 1), 5 to 8 weeks (group 2), 9 to 12 weeks (group 3), 13 to 16 weeks (group 4), 17 to 24 weeks (group 5), and longer than 24 weeks (group 6). The results are shown in **Table 2**.

The different routes of administration for anesthesia were also analyzed, with the following results: intravenous (1), inhalation (3), subcutaneous (3), intramuscular (6), intraperitoneal (33), and 3 undefined.

One of the main focuses of this SR was on the different

methods to repair an injured nerve and the materials used for that purpose. The anastomoses can be performed by approximating the nerve edges from the epineurium or the perineurium of the fascicles. Epineurial coaptation was the approximation technique used in 41 of the studies, the perineurial technique was performed in 6, a full thickness neurorrhaphy was used in one study and 3 reports did not describe the type of anastomosis used. There are only two ways to directly repair a nerve that has suffered a trauma: ETE or ETS coaptation. The number of studies that performed an ETE or ETS neurorrhaphy was 40 and 14, respectively, because some of them combined or compared both techniques. Four different techniques were used to carry out the nerve coaptation: suture, glue, laser, or mechanical. Of the 49 studies, 46 used microsuture techniques in order to perform the neurorrhaphies, 12 used glue, 8 used laser and 2 used mechanical techniques. There was only one study that did not specify the technique that they used (data not shown). The suture technique is the most common way to repair the nerve, compared to glue, laser, and mechanical methods. The size range of suture used was between 8-0 and 12-0, with 10-0 the most commonly used, and with nylon sutures being the material of choice in 70% of the studies. As previously mentioned, glues were used in 12 studies: 9 with

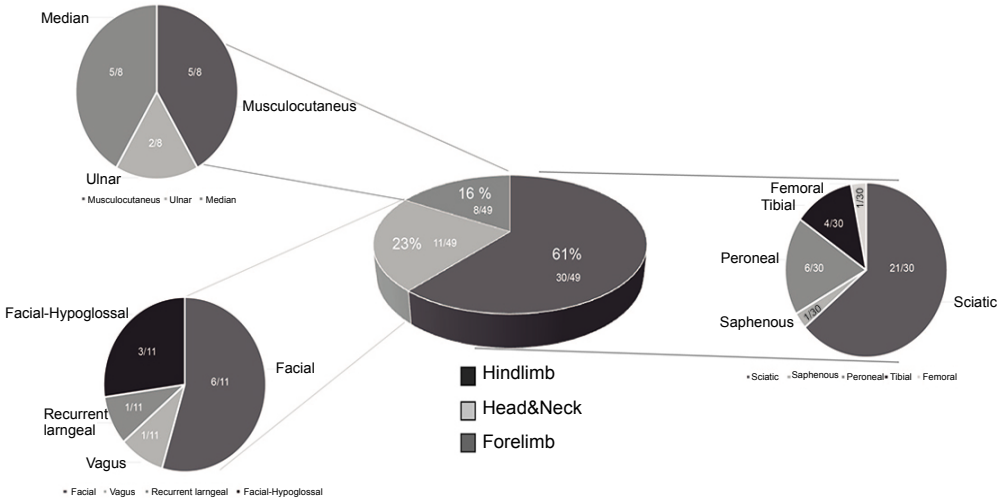

Figure 2 Types of nerves used in preclinical peripheral nerve repair studies
The nerves used in preclinical trials are divided into three groups: head & neck, hindlimb and forelimb. The frequency of the different nerves of each group is represented, in order to give a clear view of the nerves used.

Table 2 Peripheral nerve repair preclinical studies: postoperative follow-up period, number of studies and animal models

Time point	Number of studies (percentage)	Rat	Rabbit	Mouse	Guinea pig	Cat	Dog
1–4 weeks	28/89 (31.5)	Lutz et al. (2000); Tiangco et al. (2001); Suri et al. (2002); Menovsky and Beek (2003); Wieken et al. (2003); Hwang et al. (2005); Peker et al. (2005); Kokkalis et al. (2009); Kostopoulos et al. (2009); Wang et al. (2009); Fox et al. (2012); Wu et al. (2013); Adel et al. (2017)	Park et al. (2002); Dourado et al. (2004)				
5–8 weeks	18/89 (20.2)	Lutz et al. (2000); Shamir et al. (2001); Papakonstantinou et al. (2002); Suri et al. (2002); Menovsky and Beek (2003); Wu et al. (2013); Hwang et al. (2005, 2006); Peker et al. (2005); Bao et al. (2016); Bhatt et al. (2017a, b)	Park et al. (2002); Dourado et al. (2004); Hwang et al. (2008)	Félix et al. (2013)	Cho et al. (2010)		
9–12 weeks	20/89 (22.5)	Al Qattan (2000); Howard et al. (2000); Suri et al. (2002); Isla et al. (2003); Bao et al. (2017a); Choi et al. (2004); Isaac et al. (2005); Liu et al. (2005); Peker et al. (2005); Wang et al. (2009); Nunes e Silva et al. (2010, 2012); Fox et al. (2012); Omori et al. (2012); Wu et al. (2013); Fekrazad et al. (2017); Hastruck et al. (2018)	Park et al. (2002)	Tos et al. (2008)			
13–16 weeks	7/89 (7.9)	Menovsky and Beek (2001); Knox et al. (2013); Liu et al. (2018)	Beer et al. (2004); Dourado et al. (2004)				Attar et al. (2012)
17–24 weeks	6/89 (6.7)	Lutz et al. (2000); Lutz and Lidman (2005); Ozkan et al. (2005); Peker et al. (2005); Landegren et al. (2006); Fox et al. (2012)					
> 24 weeks	10/89 (11.2)	Zhang et al. (2000); Papakonstantinou et al. (2002); Peker et al. (2005); Fox et al. (2012); Papalia et al. (2012); Liu et al. (2018)	Giovanoli et al. (2000)			Hu et al. (2009)	

Frequency and percentage of publications classified by animal model and end time point of study. From the 49 studies included, there are some of them with more than one end time period, being in total 89 end time periods.

fibrin glue and 3 with cyanoacrylate. The most widely used laser in preclinical trials was CO_2, employed in 6 studies, including 1 study which compared the CO_2 laser with a potassium titanyl phosphate (KTP) laser. One study examined KTP laser effectiveness and one used a diode laser together with a protein solder. Mechanical techniques were reported in only 2 studies, 1 using titanium clips VCS® and the other using a 1.5 mm coupler to perform the nerve coaptation (data not shown).

The follow-up exams used in the studies were classified according to the type of exam. Histological analyses were performed in 43 studies and the objective of the evaluation is shown in **Figure 3A**. Meanwhile, 24 articles reported different electrophysiological examinations (**Figure 3B**). Finally the other exams that were described in 22 studies were behavioral observations of the animals. Because the target nerve in each study was not the same, these were not consistent for all studies. We therefore organized the behavioral observations according to the nerve model (**Table 3**).

Outcomes

In all studies, the primary outcome was nerve repair and regeneration, measured by axon recovery identified through histology. In addition, some studies explored muscle reinnervation by electromyography, while others recorded the behavioral observations.

Risk of bias within studies

Table 4 shows the SYRCLE's risk of bias tool (Hooijmans et al., 2014), with the information of bias extracted from the studies. The types of bias extracted were: selection bias, performance bias, detection bias, attrition bias and reporting bias. The highest risks were found in the allocation concealment (selection bias) and blinding (performance bias), with only one study that described the method of concealment and provided the information to show that the trial was effectively blind. According to detection bias, there were some studies with the outcome assessor blinded and selected the animals randomly for outcome assessment. There were no studies with high risk of bias regarding the attrition and reporting bias.

Discussion

Summary of evidence

Registration of SRs may reduce the risk of multiple reviews addressing the same question (Liberati et al., 2009) and, consequently, we attempted to register the present SR in PROSPERO, an international prospective register of SRs. However, this SR was not eligible for inclusion because the outcomes of our included studies are not directly related to human health.

There has been a gradual decrease in the number of publications in the field of direct peripheral nerve repair, which may be due to the promotion of new therapeutic techniques, such as autografts, allografts or conduits (Lovati et al., 2018), as alternative methods to reestablish the connection in a nerve gap (Eren et al., 2018).

One of the factors affecting nerve regeneration is the time lapse from damage to intervention. In 2005 and 2013, two studies addressed this issue, demonstrating that there are no notable differences between immediate nerve repair and that performed 2 weeks (Peker et al., 2005) or 4 weeks (Wu et al., 2013) after injury, with the only difference being the amount of tissue that has to be removed from the injured nerve with the passing of time. Suture techniques are the most common method used in order to repair a nerve injury. However, glue and laser techniques are becoming suitable alternatives for performing nerve coaptation (Menovsky and Beek, 2001; Wieken et al., 2003). As such, it is necessary to compare both alternatives with suturing. A number of authors have reported similar outcomes whether using glues or sutures for ETE coaptations (Menovsky and Beek, 2001; Suri et al., 2002; Dourado et al., 2004; Landegren et al., 2006; Attar et al., 2012; Félix et al., 2013; Knox et al., 2013). Yet others promote the use of glue over sutures (Adel et al., 2017), which may be due to the progress of research into glues. In 2004, the use of glues became a meaningful alternative to sutures when nerve injury occurs in confined anatomical locations (Choi et al., 2004). Also, it is possible to perform ETS neurorrhaphy with glue, obtaining no signif-

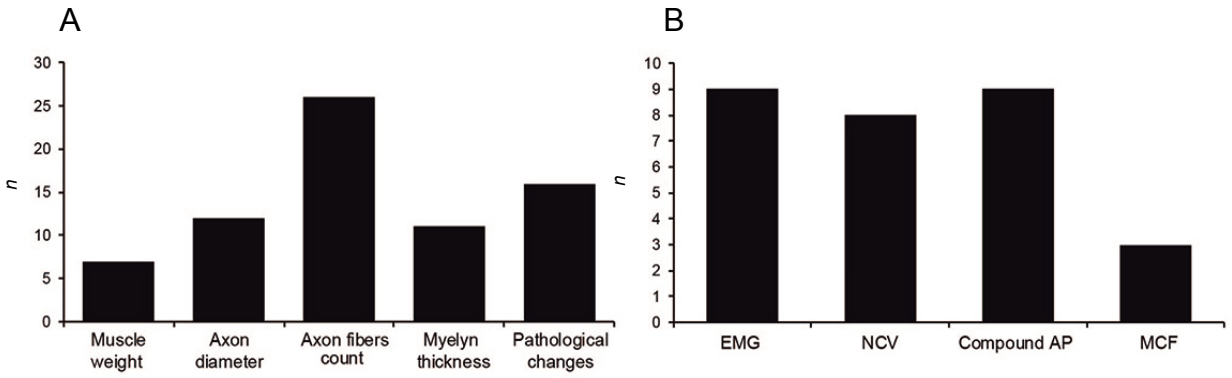

Figure 3 Postoperative assessment after peripheral nerve reconstruction.
(A) Histological parameters. (B) Electrophysiological studies. AP: Action potential; EMG: electromyography; MCF: muscle contraction force; n: number of studies; NCV: nerve conduction velocity.

Table 3 Behavioral observations and frequency classified by nerve

Nerve	Frequency	Behavioral observations	Study
Sciatic	9/22	Foot print test	Howard et al. (2000); Félix et al. (2013); Fekrazad et al. (2017)
		Sciatic functional index	Papakonstantinou et al. (2002); Félix et al. (2013)
		Walking track analysis (PL, TS, ITS)	Howard et al. (2000); Özkan et al. (2005); Fekrazad et al. (2017)
		Toe spreading test	Menovsky and Beek (2001); Özkan et al. (2005)
		Pinch reflex test	Lutz and Lidman (2005); Özkan et al. (2005)
		Limb circumference measurement and toe contracture	Özkan et al. (2005)
		Gross examination	Park et al. (2002)
		Gait analysis	Papakonstantinou et al. (2002)
		Force threshold analysis	Howard et al. (2000)
Peroneal	3/22	Walking track analysis	Nunes e Silva et al. (2010, 2012)
		Toe spreading reflex	Beer et al. (2004)
Tibial	3/22	Walking track analysis	Howard et al. (2000); Bhatt et al. (2017a)
		Force threshold analysis	Howard et al. (2000); Bhatt et al. (2017b)
		Foot print	Howard et al. (2000)
Facial-Hypoglossal	3/22	Vibrissae motor performance: whisking frequency, angle and maximal protraction, amplitude and angular velocity	Liu et al. (2018)
		Functional whisking	Knox et al. (2013)
		Vibrissae movements	Cho et al. (2010)
		Eye closure	
Musculocutaneous	5/22	Grooming test	Lutz et al. (2000); Tiango et al. (2001); Kokkalis et al. (2009); Kostopoulos et al. (2009); Bao et al. (2016)
		Grasping test	Lutz et al. (2000)
		Normal elbow flexion	Tiango et al. (2001); Kokkalis et al. (2009); Kostopoulos et al. (2009); Bao et al. (2016)
Ulnar	2/22	Grasping test	Papalia et al. (2012)
		Neurological function, gait analysis, finger pinch response and autophagy.	Isla et al. (2003)
Median	5/22	Grasping test	Tos et al. (2008); Papalia et al. (2012)
		Grooming test	Kokkalis et al. (2009); Kostopoulus et al. (2009); Bao et al. (2016)

Classification y frequency of nerves with the behavioral observation assessments described and authors that study it. IT: Intermediary toe spread; PL: print length. TS: toe spread.

icant difference in outcome compared to suture techniques (Nunes e Silva et al., 2010, 2012). Nevertheless, it is demonstrated that glues in ETS coaptation with epineurial window had better outcomes that without window (Papalia et al., 2016; Geuna et al., 2017). In a comparison between organic and inorganic glues (fibrin and cyanoacrylate, respectively), fibrin is reported to be more suitable, due to its lower induction of foreign body and inflammatory reactions (Wieken et al., 2003). Regarding lasers, the first results were similar to those obtained with sutures, but with limitations (Menovsky and Beek, 2001). With advances in technology, lasers have become a viable alternative for the performance of nerve anastomosis (Hwang et al., 2005, 2006, 2008) and, some studies now recommend lasers over suture techniques (Bhatt et al., 2017a, b; Fekrazad et al., 2017), possibly due to the increased quality of laser technology. Only one study combines the use of suture and laser, using only two stitches of different sutures to approximate the nerve ends (Menovsky and Beek, 2003), and the conclusion of this study was that polyglycolic acid (PGA) stimulates less foreign body reaction than nylon, and is best used together with a laser. Finally, laser use was combined with chitosan, improving the outcomes in nerve repair (Bhatt et al., 2017a).

In 2002, Park et al. used titanium clips and concluded that this was a faster technique with a statistically comparable outcome compared to suture neurorrhaphy. Meanwhile, in 2005, a coupler was used and reported to be a suitable alternative to sutures (Lutz and Lidman, 2005). The limitations with these approaches are the foreign body reaction, inflammatory response, uncertainty regarding endoneurium damage when using clips, and the size of the coupler. Mechanical techniques, such as clips, have been proposed to be faster rather than suturing, and could provide an alternative approach to reduce operating time when the nerve is not the only tissue affected (Park et al., 2002). However, the coupler has fewer benefits in nerve regeneration compared with suture neurorrhaphy due to its rigidity and prevention of the crisscrossing of regenerating axons (Lutz and Lidman, 2005).

In the other articles, the suture technique was used to perform the coaptations, and the variability between them is extensive due to the size of the suture and the material used. The standard suture is size 10-0 and made of nylon, but alternative sizes and materials may be used. As the rat was the most commonly used animal model, and the size of rat nerves are very small, use of the smallest suture available increases the ease of the procedure. Suture size 12-0 was used in mice for a similar reason. Regarding types of neurorrhaphy, ETS gave worse results than ETE (Lutz et al., 2000; Liu et al., 2005, 2018; Kokkalis et al., 2009; Papalia et al., 2012), but remains a valid option when tension cannot

Table 4 Summary of SYRCLE's risk of bias

Study	Random sequence analysis	Baseline characteristics	Allocation concealment	Random housing	Blinding	Random outcome assessmen	Binding of outcome assessment	Incomplete outcome data	Selective reporting
Adel et al. (2017)	High	Low	High	High	High	High	High	Low	Low
Al-Qattan (2000)	High	Low	High	High	High	Low	High	Low	Low
Attar et al. (2012)	High	Low	High	High	High	High	High	Low	Low
Bao et al. (2016)	Low	Low	High	High	High	Low	Low	Low	Low
Beer et al. (2004)	High	Low	High	Low	High	High	High	Low	Low
Bhatt et al. (2017a)	High	Low	High	Low	High	High	Low	Low	Low
Bhatt et al. (2017b)	High	Low	High	Low	High	High	Low	Low	Low
Cho et al. (2010)	Low	Low	High	High	High	High	High	Low	Low
Choi et al. (2004)	High	Low	High	High	High	High	High	Low	Low
Dourado et al. (2004)	High	Low	High	Low	High	High	High	Low	Low
Fekrazad et al. (2017)	Low	High	High	Low	High	High	High	Low	Low
Felix et al. (2013)	High	Low	High	Low	High	High	Low	Low	Low
Fox et al. (2012)	Low	Low	High	Low	High	Low	Low	Low	Low
Giovanoli et al. (2000)	High	Low	High	High	High	High	High	Low	Low
Hastruck et al. (2018)	Low	Low	High	Low	High	High	High	Low	Low
Howard et al. (2000)	High	Low	High	High	High	High	High	Low	Low
Hu et al. (2009)	High	High	High	High	High	High	High	Low	Low
Hwang et al. (2005)	High	Low	High	High	High	High	High	Low	Low
Hwang et al. (2006)	High	Low	High	High	High	High	High	Low	Low
Hwang et al. (2008)	High	Low	High	High	High	High	High	Low	Low
Isaacs et al. (2005)	High	Low	High	Low	High	High	High	Low	Low
Isla et al. (2003)	High	Low	High	High	High	High	Low	Low	Low
Knox et al. (2013)	Low	Low	High	Low	High	High	High	Low	Low
Kokkalis et al. (2009)	Low	Low	High	Low	High	High	Low	Low	Low
Kostopoulos et al. (2009)	High	Low	High	Low	High	High	Low	Low	Low
Landegren et al. (2006)	High	Low	High	Low	High	Low	High	Low	Low
Liu et al. (2005)	Low	Low	High	High	High	High	Low	Low	Low
Liu et al. (2018)	Low	Low	High	High	High	High	Low	Low	Low
Lutz et al. (2000)	High	Low	High	Low	High	High	High	Low	Low
Lutz and Lidman (2005)	High	Low	High	Low	High	High	High	Low	Low
Menovsky and Beek (2001)	High	Low	High	Low	High	High	High	Low	Low
Menovsky and Beek (2003)	High	Low	High	Low	High	High	High	Low	Low
Nunes e Silva et al. (2010)	High	Low	High	High	High	High	Low	Low	Low
Nunes e Silva et al. (2012)	Low	Low	High	High	High	High	High	Low	Low
Omori et al. (2012)	Low	Low	High	Low	High	High	High	Low	Low
Ozkan et al. (2005)	High	Low	High	Low	High	High	Low	Low	Low
Papakonstantinou et al. (2012)	Low	Low	High	Low	High	High	Low	Low	Low
Papalia et al. (2012)	Low	Low	High	Low	High	Low	Low	Low	Low
Park et al. (2002)	Low	Low	High	Low	High	High	Low	Low	Low
Peker et al. (2005)	High	Low	High	High	High	High	High	Low	Low
Shamir et al. (2001)	Low	Low	Low	High	Low	Low	Low	Low	Low
Suri et al. (2002)	High	Low	High	High	High	High	High	Low	Low
Tiangco et al. (2001)	Low	Low	High	Low	High	High	Low	Low	Low
Tos et al. (2008)	High	High	High	Low	High	Low	High	Low	Low
Wang et al. (2009)	High	High	High	Low	High	High	Low	Low	Low
Wieken et al. (2003)	High	Low	High	High	High	High	High	Low	Low
Wu et al. (2013)	Low	Low	High	Low	High	High	High	Low	Low
Yan et al. (2002)	High	Low	High	Low	High	High	Low	Low	Low
Zhang et al. (2000)	Low	Low	High	Low	High	Low	High	Low	Low

Author's judgements about all type of bias for each publication reviewed. Selection bias (sequence generation, baseline characteristics and allocation concealment), performance bias (random housing and blinding), detection bias (random outcome assessment and blinding), attrition bias (incomplete outcome data) and reporting bias (Selective outcome reporting).

be avoided while attempting to perform an ETE coaptation (Zhang et al., 2000; Isaacs et al., 2005). In addition, the outcomes of ETS anastomoses can be improved using ancillary treatments. These compounds used in conjunction with nerve coaptations were: anti-adhesion barrier gel (Isla et al., 2003), oral administration of creatine (Ozkan et al., 2005), neuronal nitric oxide synthase (Wang et al., 2009), platelet rich plasma and mesenchymal stem cells (Cho et al., 2010),

and rat or human amniotic membrane (Hasturk et al., 2018), insulin-like growth factor-I (Tiangco et al., 2001), acetyl-L carnitine (Kostopoulos et al., 2009) or irradiation of the spinal cord with an low power laser (Shamir et al., 2001). It is demonstrated that acetyl-L carnitine prevent the sensory neuronal loss after peripheral nerve injury and it has neuroprotective effect (Wilson et al., 2007). There are currently no clear improvements in nerve regeneration using pharmacological methods, but potential candidates for enhancing nerve regeneration could emerge in the foreseeable future (Panagopoulos et al., 2017).

Rats are the most commonly used animals for preclinical trials of direct peripheral nerve repair. Although there is insufficient evidence to support the use of other species, such as dog, cat, guinea pig or mouse (Tos et al., 2008; Hu et al., 2009; Cho et al., 2010; Attar et al., 2012; Félix et al., 2013), the rabbit may provide a possible alternative to rat (Giovanoli et al., 2000; Park et al., 2002; Beer et al., 2004; Dourado et al., 2004; Hwang et al., 2008), with their larger and thicker nerves. However, the relatively low cost of rats compared to these alternative models presents a significant advantage. Rats have a brachial plexus structure very similar to human beings (Bobkiewicz et al., 2017), the experimental results using rodent forelimb models are more commonly translated to operating theaters (Tos et al., 2008), but it is necessary to take into account that rodents have a faster regenerating capacity compared to humans (Zhang et al., 2000; Wu et al., 2013). On the other hand, rabbits have more active masseter movements, jaw development, and bigger size and weight than rats, thus they are a really good animal model studying head and neck nerves as facial (Hwang et al., 2008). Despite the big number of studies using rats as animal model in hindlimb nerves, it is not the best model to investigate because it translation to human beings has shown to be unreliable for nerve regeneration (Kaplan et al., 2015). However rats should only be employed in questions about basic science, where background data strongly supports a model's validity. Finally, the number of nerve fibers and nerve size of dogs are close to human ones, making this animal model perfect to practice the nerve coaptation in similar conditions than in clinical practice (Attar et al., 2012).

The biggest limitation of the murine models in nerve repair is the length of the nerves and the difficulty to avoid tension during their repair. For these reasons, the rodent may be a poor option when studying nerve regeneration in a gap, conduit or graft model (Félix et al., 2013; Griffin et al., 2014; Kaplan et al., 2015).

Follow-up exams are also important to determine the extent of regeneration. Histology is possibly the most important assessment, but it can only be analyzed once, at the end of the study. In order to evaluate the progression of nerve regeneration, it is beneficial to be able to examine the model at different time points in the same study (Mackinnon et al., 1991). Electrophysiological analysis can reveal whether or not there is nerve recovery while the animal is still alive, but only concerning motor nerves (Kanaya et al., 1996). Behavioral observations yield a lot of information about motor function recovery, but this can be dependent on the nerve of interest. For example, the motor function of median nerve can be study using the grasping test, but walking track analysis is the most used examination in order to determine the sciatic nerve motor function (Papalia et al., 2003). In this context, the sciatic nerve is the gold standard of hindlimb nerves; the facial nerve, for head and neck nerves; and the median and musculocutaneous nerves in the forelimbs (de Medinaceli et al., 1982; Berg and Kleinfeld, 2003).

Limitations

Outcome level

The present SR combines data across studies with the goal of determining suitable animal models for any peripheral nerve studies. The main limitations of this review are the variety of nerve models and the large variability in the length of the studies.

Study and review level

Our work has a number of limitations, including the choice of language, because there may be more articles that could be included according to the inclusion criteria, but written in languages other than English. The use of anastomosis as search word instead of coaptation has to be taken into account, because it is a more appropriate terminology for nerve repair and possibly many studies could be missed in our review.

Conclusions

Between the years 2000 and 2018, the number of publications addressing preclinical trials of direct nerve repair has decreased, implying that researchers have been focusing on other fields of nerve repair. Comparing the different techniques currently available, the suture and glue methods are effective options, because their use results in promising outcomes, but the laser method is still being debated. Depending on the nerve of interest, the animal model may vary, for example rats are more indicated for studying forelimb nerves while rabbits represent a better option for facial nerve. To study nerve recovery, the protocol should include a histological study, an electrophysiological analysis and the observation of behavioral parameters appropriate to the nerve of interest.

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References

Adel M, Abdo Elgamal D, Bakry R, Abdelkader M, Elshazly M, Kamel A (2017) Suture versus fibrin glue microneural anastomosis of the femoral nerve in Sprague Dewly rat model. A comparative experimental assessment of the clinical, histological and statistical features. Acta Chir Plast 59:65-71.

Al-Qattan MM (2000) Prevention and treatment of painful neuromas of the superficial radial nerve by the end-to-side nerve repair concept: An experimental study and preliminary clinical experience. Microsurgery 20:99-104.

Angius D, Wang H, Spinner RJ, Gutierrez-Cotto Y, Yaszemski MJ, Windebank AJ (2012) A systematic review of animal models used to study nerve regeneration in tissue-engineered scaffolds. Biomaterials 33:8034-8039.

Attar BM, Zalzali H, Razavi M, Ghoreishian M, Rezaei M (2012) Effectiveness of fibrin adhesive in facial nerve anastomosis in dogs compared with standard microsuturing technique. J Oral Maxillofac Surg 70:2427-2432.

Bao Q, Xiao C, Wang T, Gu Y (2016) Novel atraumatic end-to-side repair model exhibits robust collateral sprouting independent of donor fiber injury. Plast Reconstr Surg 137:523-533.

Beer GM, Burg D, Zehnder A, Seifert B, Steurer M, Grimaldi H, Meyer VE (2004) Functional, electrophysiologic, and morphometric evaluation of nerve regeneration from coaptation on regenerated nerve fibers: experimental study in rabbits. J Reconstr Microsurg 20:159-166.

Berg RW, Kleinfeld D (2003) Rhythmic whisking by rat: retraction as well as protraction of the vibrissae is under active muscular control. J Neurophysiol 89:104-117.

Bhatt NK, Khan TR, Mejias C, Paniello RC (2017a) Nerve transection repair using laser-activated chitosan in a rat model. Laryngoscope 127:E253-257.

Bhatt NK, Mejias C, Kallogjeri D, Gale DC, Park AM, Paniello RC (2017b) Potassium titanyl phosphate laser welding following complete nerve transection. Laryngoscope 127:1525-1530.

Birch R, Misra P, Stewart MP, Eardley WG, Ramasamy A, Brown K, Shenoy R, Anand P, Clasper J, Dunn R, Etherington J (2012) Nerve injuries sustained during warfare: part I--Epidemiology. J Bone Joint Surg Br 94:523-528.

Bobkiewicz A, Cwykiel J, Siemionow M (2017) Anatomic variations of brachial and lumbosacral plexus models in different rat strains: anatomic variations of brachial and lumbosacral plexus models. Microsurgery 37:327-333.

Cho HH, Jang S, Lee SC, Jeong HS, Park JS, Han JY, Lee KH, Cho YB (2010) Effect of neural-induced mesenchymal stem cells and platelet-rich plasma on facial nerve regeneration in an acute nerve injury model. Laryngoscope 120:907-913.

Choi BH, Kim BY, Huh JY, Lee SH, Zhu SJ, Jung JH, Cho BP (2004) Microneural anastomosis using cyanoacrylate adhesives. Int J Oral Maxillofac Surg 33:777-780.

Daly WT, Yao L, Abu-rub MT, O'Connell C, Zeugolis DI, Windebank AJ, Pandit AS (2012) The effect of intraluminal contact mediated guidance signals on axonal mismatch during peripheral nerve repair. Biomaterials 33:6660-6671.

de Medinaceli L, Freed WJ, Wyatt RJ (1982) An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks. Exp Neurol 77:634-643.

Dourado EP, Valmaseda-Castellon E, Gay-Escoda C (2004) Facial nerve repair with epineural suture and anastomosis using fibrin adhesive: An experimental study in the rabbit. J Oral Maxillofac Surg 62:1524-1529.

Dvali L, Mackinnon S (2007) The role of microsurgery in nerve repair and nerve grafting. Hand Clin 23:73-81.

Eren A, Atalar H, Seymen CM, Alpaslan Pınarlı F, Take Kaplanoglu G, Turanlı S (2018) Sutureless approach with vein grafts and mesenchymal stem cells in primary nerve repair: Functional and immunohistological results. Microsurgery 38:780-789.

Fekrazad R, Mortezai O, Pedram M, Kalhori KA, Joharchi K, Mansoori K, Ebrahimi R, Mashhadiabbas F (2017) Transected sciatic nerve repair by diode laser protein soldering. J Photochem Photobiol B 173:441-447.

Félix SP, Pereira Lopes FR, Marques SA, Martinez AM (2013) Comparison between suture and fibrin glue on repair by direct coaptation or tubulization of injured mouse sciatic nerve. Microsurgery 33:468-477.

Fox IK, Brenner MJ, Johnson PJ, Hunter DA, Mackinnon SE (2012) Axonal regeneration and motor neuron survival after microsurgical nerve reconstruction. Microsurgery 32:552-562.

Geuna S, Papalia I, Ronchi G, d'Alcontres F, Natsis K, Papadopulos N, Colonna M (2017) The reasons for end-to-side coaptation: how does lateral axon sprouting work? Neural Regen Res 12:529-533.

Giovanoli P, Koller R, Meuli-Simmen C, Rab M, Haslik W, Mittlbock M, Meyer VE, Frey M (2000) Functional and morphometric evaluation of end-to-side neurorrhaphy for muscle reinnervation. Plast Reconstr Surg 106:383-392.

Griffin MF, Malahias M, Hindocha S, Wasim S K (2014) Peripheral nerve injury: principles for repair and regeneration. Open Orthop J 8:199-203.

Hasturk AE, Yilmaz ER, Hayirli N, Kayalar AE, Akyildiz S, Gokce EC, Akcay I, Evirgen O, Bakir A (2018) Stereologic and ultrastructural comparison of human and rat amniotic membrane wrapping for rat sciatic nerve repair. J Clin Neurosci 57:157-161.

Hooijmans CR, Rovers MM, de Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW (2014) SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol 14:43.

Howard CS, Blakeney DC, Medige J, Moy OJ, Peimer CA (2000) Functional assessment in the rat by ground reaction forces. J Biomech 33:751-757.

Hu ME, Tyan YS, Hsu PW, Hsu JC, Chang HM, Ling EA, Lan CT (2009) Ultrastructural observations on the progress of nerve degeneration and regeneration at the suture site following vagal-hypoglossal nerve coaptation in cats. Cells Tissues Organs 190:230-245.

Huckhagel T, Nüchtern J, Regelsberger J, Gelderblom M, Lefering R, TraumaRegister DGU® (2018a) Nerve trauma of the lower extremity: evaluation of 60,422 leg injured patients from the TraumaRegister DGU® between 2002 and 2015. Scand J Trauma Resusc Emerg Med 26:40.

Huckhagel T, Nüchtern J, Regelsberger J, Lefering R, TraumaRegister DGU (2018b) Nerve injury in severe trauma with upper extremity involvement: evaluation of 49,382 patients from the TraumaRegister DGU® between 2002 and 2015. Scand J Trauma Resusc Emerg Med 26:76.

Hwang K, Kim SG, Kim DJ (2006) Facial-hypoglossal nerve anastomosis using laser nerve welding. J Craniofac Surg 17:687-691.

Hwang K, Kim SG, Kim DJ (2008) Hypoglossal-facial nerve anastomosis in the rabbits using laser welding. Ann Plast Surg 61:452-456.

Hwang K, Kim SG, Kim DJ, Lee CH (2005) Laser welding of rat's facial nerve. J Craniofac Surg 16:1102-1106.

Isaacs J, Allen D, Chen LE, Nunley J (2005) Reverse end-to-side neurotization. J Reconstr Microsurg 21:43-50.

Isla A, Martinez JR, Perez-Lopez C, Perez Conde C, Morales C, Budke M (2003) A reservable antiadhesion barrier gel reduces the perineural adhesions in rats after anastomosis. J Neurosurg Sci 47:195-200

Kanaya F, Firrell JC, Breidenbach WC (1996) Sciatic function index, nerve conduction tests, muscle contraction, and axon morphometry as indicators of regeneration. Plast Reconstr Surg 98:1264-1274.

Kaplan HM, Mishra P, Kohn J (2015) The overwhelming use of rat models in nerve regeneration research may compromise designs of nerve guidance conduits for humans. J Mater Sci Mater Med 26:226.

Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol 8:e1000412.

Knox CJ, Hohman MH, Kleiss IJ, Weinberg JS, Heaton JT, Hadlock TA (2013) Facial nerve repair: fibrin adhesive coaptation versus epineurial suture repair in a rodent model. Laryngoscope 123:1618-1621.

Kokkalis ZT, Soucacos PN, Terzis JK (2009) Effect of donor nerve injury distal to an end-to-side neurorrhaphy model. J Reconstr Microsurg 25:295-306.

Kostopoulos VK, Davis CL, Terzis JK (2009) Effects of acetylo-l-carnitine in end-to-side neurorrhaphy: a pilot study. Microsurgery 29:456-463.

Kouyoumdjian JA (2006) Peripheral nerve injuries: a retrospective survey of 456 cases. Muscle Nerve 34:785-788.

Landegren T, Risling R, Brage A, Persson JKE (2006) Long-term results of peripheral nerve repair: A comparison of nerve anastomosis with ethyl-cyanoacrylate and epineural sutures. Scand J Plast Reconstr Surg Hand Surg 40:65-72.

Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, Clarke M, Devereaux PJ, Kleijnen J, Moher D (2009) The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med 6:e1000100.

Liu HJ, Dong MM, Chi FL (2005) Functional, remobilization evaluation of the paralyzed vocal cord by end-to-side neurorrhaphy in rats. Laryngoscope 115:1418-1420.

Liu P, Zhang Z, Liao C, Zhong W, Li P, Zhang W (2018) Dynamic quantitative assessment of motor axon sprouting after direct facial-hypoglossal end-to-side neurorrhaphy in rats. J Reconstr Microsurg 34:436-445.

Lovati AB, D'Arrigo D, Odella S, Tos P, Geuna S, Raimondo S (2018) Nerve repair using decellularized nerve grafts in rat models. a review of the literature. Front Cell Neurosci 12:427.

Lutz BS, Lidman D (2005) Morphological and functional evaluation of leg-muscle reinnervation after coupler coaptation of the divided rat sciatic nerve. Microsurgery 25:235-240.

Lutz BS, Ma SF, Chuang DC, Wei FC (2000) Role of the target in end-to-side neurorrhaphy: reinnervation of a single muscle vs. multiple muscles. J Reconstr Microsurg 16:443-448.

Mackinnon SE, Dellon AL, O'Brien JP (1991) Changes in nerve fiber numbers distal to a nerve repair in the rat sciatic nerve model. Muscle Nerve 14:1116-1122.

Menovsky T, Beek JF (2001) Laser, fibrin glue, or suture repair of peripheral nerves: a comparative functional, histological, and morphometric study in the rat sciatic nerve. J Neurosurg 95:694-699.

Menovsky T, Beek JF (2003) Carbon dioxide laser-assisted nerve repair: Effect of solder and suture material on nerve regeneration in rat sciatic nerve. Microsurgery 23:109-116.

Mohanty CB, Bhat DI, Devi BI (2019) Use of animal models in peripheral nerve surgery and research. Neurol India 67:S100-105.

Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 6:e1000097.

Nunes e Silva D, Brochado Antoniolli da Silva ACM, Aydos RD, Viterbo F, Jardim Cury Pontes ER, Odashiro DN, de Castro RJ, Augusto DG (2012) Nerve growth factor with fibrin glue in end-to-side nerve repair in rats. Acta Cir Bras 27:325-332.

Nunes e Silva D, Coelho J, Frazilio F de O, Odashiro AN, Camillo de Carvalho P de T, Jardim Cury Pontes ER, Vargas AF, Rosseto M, Antoniolli da Silva AB (2010) End-to-side nerve repair using fibrin glue in rats. Acta Cir Bras 25:158-162.

Omori M, Sakakibara S, Hashikawa K, Terashi H, Tahara S, Sugiyama D (2012) Comparison of reinnervation for preservation of denervated muscle volume with motor and sensory nerve: An experimental study. J Plast Reconstr Aesthet Surg 65:943-949.

Ozkan O, Duman O, Haspolat S, Ozgentas HE, Dikici MB, Gurer I, Gungor HA, Gokhan AG (2005) Effect of systemic creatine monohydrate supplementation on denervated muscle during reinnervation: Experimental study in the rat. J Reconstr Microsurg 21:573-579.

Panagopoulos GN, Megaloikonomos PD, Mavrogenis AF (2017) The present and future for peripheral nerve regeneration. Orthopedics 40:e141-156.

Papakonstantinou KC, Shiamishis G, Bates M, Terzis JK (2002) Distraction osteogenesis using IGF-I after nerve microreconstruction. J Reconstr Microsurg 18:401-410.

Papalia I, Magaudda L, Righi M, Ronchi G, Viano N, Geuna S, Colonna MR (2016) Epineurial window is more efficient in attracting axons than simple coaptation in a sutureless (cyanoacrylate-bound) model of end-to-side nerve repair in the rat upper limb: functional and morphometric evidences and review of the literature. PLoS One 11:e0148443.

Papalia I, Ronchi G, Muratori L, Mazzucco A, Magaudda L, Geuna S (2012) Direct muscle neurotization after end-to-end and end-to-side neurorrhaphy An experimental study in the rat forelimb model. Neural Regen Res 7:2273-2278.

Papalia I, Tos P, Stagno d'Alcontres F, Battiston B, Geuna S (2003) 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 127:43-47.

Park JW, Lee KS, Kim SK, Park JH, Hong JS, Oh KJ (2002) Rapid neurorrhaphy with titanium clips. Microsurgery 22:386-390.

Peker F, Solako lu C, Yuksel F, Kutlay M (2005) Effects of time lapse on results of partial nerve injury repair. J Reconstr Microsurg 21:145-149.

Robinson LR (2000) Traumatic injury to peripheral nerves. Muscle Nerve 23:863-873.

Robinson LR (2004) traumatic injury to peripheral nerves. Suppl Clin Neurophysiol 57:173-186.

Sanders FK, Young JZ (1942) The degeneration and re-innervation of grafted nerves. J Anat 76:143-166.7.

Shamir MH, Rochkind S, Sandbank J, Alon M (2001) Double-blind randomized study evaluating regeneration of the rat transected sciatic nerve after suturing and postoperative low-power laser treatment. J Reconstr Microsurg 17:133-138.

Siemionow M, Brzezicki G (2009) Current techniques and concepts in peripheral nerve repair. In: Essays on peripheral nerve repair and regeneration (Geuna S, Tos P, Battiston B, eds), pp 141-172. San Diego: Elsevier Academic Press Inc.

Smith JW (1964) Microsurgery of peripheral nerves. Plast Reconstr Surg 33:317-329.

Suri A, Mehta VS, Sarkar C (2002) Microneural anastomosis with fibrin glue: An experimental study. Neurol India 50:23-26.

Tiangco DA, Papakonstantinou KC, Mullinax KA, Terzis JK (2001) IGF-I and end-to-side nerve repair: a dose-response study. J Reconstr Microsurg 17:247-256.

Tos P, Ronchi G, Nicolino S, Audisio C, Raimondo S, Fornaro M, Battiston B, Graziani A, Perroteau I, Geuna S (2008) Employment of the mouse median nerve model for the experimental assessment of peripheral nerve regeneration. J Neurosci Methods 169:119-127.

Wang SM, Tsai HP, Huang JJ, Huang HC, Lin JL, Liu PH (2009) Inhibition of nitric oxide synthase promotes facial axonal regeneration following neurorrhaphy. Exp Neurol 216:499-510.

Wieken K, Angioi-Duprez K, Lim A, Marchal L, Merle M (2003) Nerve anastomosis with glue: comparative histologic study of fibrin and cyanoacrylate glue. J Reconstr Microsurg 19:17-20.

Wilson AD, Hart A, Brännström T, Wiberg M, Terenghi G (2007) Delayed acetyl-L-carnitine administration and its effect on sensory neuronal rescue after peripheral nerve injury. J Plast Reconstr Aesthet Surg 60:114-118.

Wu P, Spinner RJ, Gu Y, Yaszemski MJ, Windebank AJ, Wang H (2013) Delayed repair of the peripheral nerve: A novel model in the rat sciatic nerve. J Neurosci Methods 214:37-44.

Yan YH, Yan JG, Sanger JR, Zhang LL, Riley DA, Matloub HS (2002) Nerve repair at different angles of attachment: Experiment in rats. Journal of Reconstructive Microsurgery 18:703-708.

Zhang Z, Soucacos PN, Beris AE, Bo J, Ioachim E, Johnson EO (2000) Long-term evaluation of rat peripheral nerve repair with end-to-side neurorrhaphy. J Reconstr Microsurg 16:303-311.

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