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REVIEW ARTICLE

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To reverse or not to reverse? A systematic review of autograft polarity on functional outcomes following peripheral nerve repair surgery

S. E. Roberts, B.A.[1,2*] | S. Thibaudeau, M.D.[3*] | J. C. Burrell, M.S.[1,2] | E. L. Zager, M.D.[1] | D. K. Cullen, Ph.D.[1,2] | and L. S. Levin, M.D.[3]

[1]Department of Neurosurgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

[2]Michael J. Crescenz Veterans Affairs Medical Center, Philadelphia, Pennsylvania

[3]Division of Plastic Surgery and Department of Orthopedic Surgery, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania

Correspondence
S. Thibaudeau, MD, 3737 Market St Suite 600, Philadelphia, PA 19104.
Email: sthiba@gmail.com

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Background: The literature describing the best clinical practice for proximal–distal autograft orientation, otherwise known as nerve graft polarity, is inconsistent. With existing disparities in the peripheral nerve literature, the clinical question remains whether reversing nerve autograft polarity bears an advantage for nerve regeneration.

Methods: A comprehensive review of the literature using Embase and PubMed databases (1940–June 2015) was performed to retrieve all original articles on the effects of nerve autograft polarity on nerve regeneration and functional recovery following primary repair of peripheral nerve defects.

Results: The initial database search yielded 318 titles. Duplicate exclusion, title review and full text review yielded six articles which directly compared nerve autograft polarity. Histological, morphometric, electrophysiological, and behavioral outcomes were reviewed. All retained articles were animal studies, of which none demonstrated significant differences in outcomes between the normal and reversed polarity groups. A reversed graft may ensure that regenerating nerve fibers are not lost at branching points, however this may not translate into improved function.

Conclusion: There is insufficient data to suggest that nerve autograft polarity has an impact on nerve regeneration and functional outcomes.

1 | INTRODCUTION

Nerve autograft has long been considered the gold standard for repair of mixed nerve injuries and nerve gaps with a deficit >3 cm (Deal, Griffin, & Hogan, 2012). Surgeons must consider the etiology of nerve injury, time since injury, gap size, nerve diameter, and personal preference when selecting the nerve repair technique with a single-stranded, cable, or vascularized autograft (Grinsell & Keating, 2014 ; Griffin, Hogan, Chhabra, & Deal, 2013; Mackinnon & Dellon, 1990).

Advances in peripheral nerve research have furthered our understanding of factors that influence nerve regeneration including precise microsurgical techniques, the time of repair relative to the time of injury, mechanism of injury, distance between the proximal lesion and distal end-target, internal nerve topography, fascicular realignment, and matching the diameter of the donor graft to that of the nerve stump.

Many surgeons anecdotally reverse autograft polarity with the intent of improving nerve regeneration by mitigating potential misrouting effects of arborization. In our search of the literature we found varied recommendations. Anderl recommends that nerve grafts should maintain their proximal–distal orientation so that branches may play a useful role in leading axons towards target muscle (Anderl, 1977). O'Brien recommends reversal of the nerve graft (O'Brien & Morrison, 1987), while Sunderland and Millesi state that reversal will not enhance regeneration (Sunderland, 1992; Millesi, 1987); however, Anderl, O'Brien, Millesi, and Sunderland provide limited clinical or basic science evidence to support their preferred technique (Anderl, 1977; O'Brien & Morrison, 1987; Sunderland, 1992; Millesi, 1987).

Though often accepted as fact, our search of the literature has revealed a paucity of studies describing nerve polarity and a blend of

*Equivalent first co-authors.

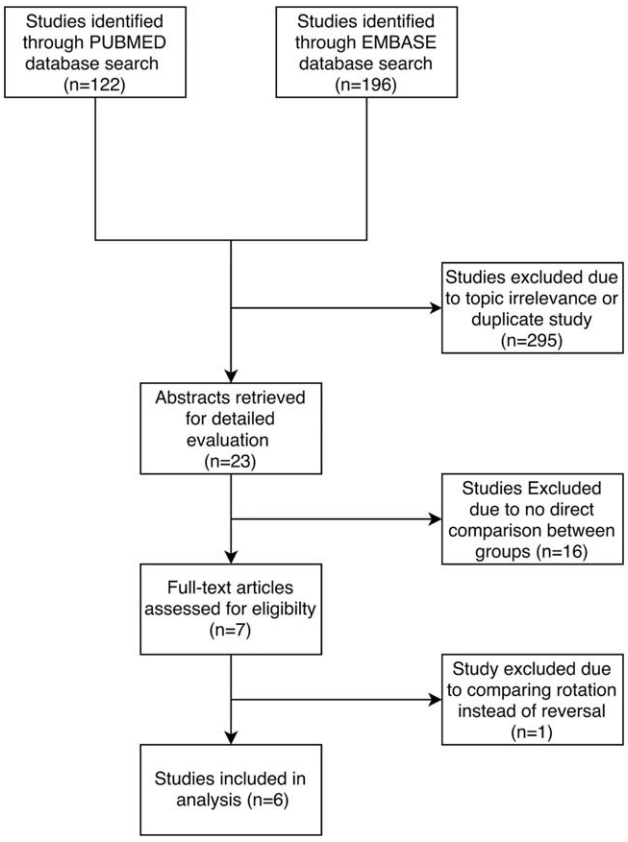

FIGURE 1 Flowchart of included and excluded studies

anecdotal clinical evidence supporting the effects of polarity reversal. We therefore sought to clarify the historical and scientific basis for reversal of autograft polarity through a systematic review of the literature.

2 | METHODS

2.1 | Search strategy

A comprehensive review of the literature was conducted in the Embase and PubMed database to retrieve all published original articles that studied the effects of nerve autograft polarity for peripheral nerve defects. Database subject headings "nerve graft polarity", "nerve graft orientation", and "nerve graft reversal" were used to identify all original articles published between January 1940 and June 2015 (Figure 1).

2.2 | Article eligibility

The inclusion criteria for this study were articles that were (1) peer-reviewed comparative studies, such as randomized controlled trials and observational studies; (2) direct comparisons of nerve graft orientation: normal proximal–distal versus reversed orientation; (3) evaluations of the effect of orientation with functional, histologic, or clinical metrics. Three reviewers (J.B., S.R., and S.T.) independently scanned the retrieved abstracts and evaluated the potential relevance. The reviewers were able to achieve consensus in article relevancy. An overview of the methods and findings for the included studies are reported in Tables 1 and 2, respectively.

2.3 | Classification and outcomes measures

In this review, included studies evaluated the effect of nerve graft orientation in animal models. We did not identify human or cadaveric studies that met our inclusion criteria. The included studies were divided into four broad categories based on their outcome measures: (1) gross measurements; (2) histopathology and morphometry; (3) electrophysiological recovery; (4) behavioral/functional assessment.

Within these broad categories variables assessed included conduction velocity, action potential amplitude, histology, morphometry, electron microscopy, axon tracing, sciatic functional index, outgrowth, and muscle weight (Table 1).

2.4 | Data extraction and analysis

The following data were extracted from each primary article and used for descriptive comparisons: author, year, sample size, nerve, animal model, study design, result time points, study results. All data are summarized descriptively.

3 | RESULTS

The literature search identified 318 potential articles. After applying the inclusion criteria, six articles were deemed relevant and included in final analysis. All studies included in our review investigated the effects of autograft polarity on nerve regeneration in rat or rabbit models. In all animal models, the autograft under study came from the same peripheral nerve model; that is, a nerve segment was cut and orientation was reversed. One study identified in the literature search examined nerve rotation, which is as defined as 180 degree rotation around the nerve's longitudinal axis, in which the proximal and distal stumps remain at the same ends. This study was excluded since it did not compare reversal of the proximal and distal stumps.

Further, the outcome measures in all six studies were based on morphometric changes and relevant histopathology. Behavioral and electrophysiology outcomes were also assessed in some studies (Table 2) (Ansselin & Davey, 1993; Nakatsuka et al., 2002; Sotereanos et al., 1992; Stromberg, Vlastou, & Earle, 1979).

In 1943, Sanders and Young used a 2 cm peroneal nerve rabbit model to examine the effects of autograft polarity on regeneration outgrowth ($n = 63$) (Sanders & Young, 1943). At 15 and 25 days post-op animals were sacrificed for nerve histopathological examination and qualitative assessment. This study reported that the axonal outgrowth distance was not significantly different between normal and reversed polarity grafts.

Stromberg et al. reported in 1979 that there were no significant differences in nerve conduction or histology at six months post-repair using a 1 cm lesion rodent model comparing nerve polarity ($n = 40$) Stromberg, et al, 1979. Based on these findings, Stromberg et al.

TABLE 1 Characteristics of included studies

Study	Sample size	Model	Study design	Peripheral nerve	Assessment modality	Follow-up
Stromberg et al., 1979	32	Sprague-Dawley rats	Control group ($n = 16$), treatment reversal group ($n = 16$)	Sciatic	Electrophysiology, Histopathology	6 mo
Ansselin & Davey, 1988	40	Male rats (species unspecified)	Un-operated control group ($n = 20$), operated control group ($n = 10$), treatment reversal group ($n = 10$)	Fibular/ Tibial	Electron microscopic, Histopathology	2 mo
Ansselin & Davey, 1993	62	Winstar rats	Un-operated control group ($n = 10$), operated controls ($n = 26$), treatment reversal group ($n = 26$)	Sciatic	Behavioral/ functional, Electrophysiology	12 mo
Sotereanos et al., 1992	60	Sprague-Dawley rats	Control group ($n = 20$), treatment reversal group ($n = 20$), treatment rotation group ($n = 20$)	Sciatic	Behavioral/ functional, Histopathology	4 mo
Nakesutura et al., 2002	12	Japanese white rabbits	Control group ($n = 6$), treatment reversal group ($n = 6$)	Common peroneal	Electrophysiology, Histopathology, Muscle weight	6 mo
Sanders & Young, 1943	63	Rabbit (species unspecified)	Groups unspecified	Tibial	Histopathology	25 days

TABLE 2 Results of included studies

Study	Histopathology & morphometry	Gross pathology	Electrophysiology	Behavioral/ functional
Stromberg et al., 1979[11]	No difference	N/A	No difference in conduction velocity or amplitude	N/A
Ansselin & Davey, 1988[14]	Normal polarity CSA < reversed in distal nerve and distal nerve stump ($P = .002$); no difference in mean diameter of myelinated axons or myelin thickness	N/A	N/A	N/A
Ansselin & Davey, 1993[9]	EM no difference; CSA of normal orientation nerve was less than the reverse orientation ($P = .008$); axon count was higher in reversed grafts ($P = .01$)	N/A	Mean conduction velocity of reverse orientation group was faster ($P = .047$)	No difference in SFI
Sotereanos et al., 1992[10]	No difference	N/A	N/A	No difference in SFI
Nakesutura et al., 2002[12]	No difference	No difference in weight of AT & EDL ($P = .21$)	No difference in motor nerve conduction velocity ($P = .65$)	N/A
Sanders & Young, 1943[13]	No difference	N/A	N/A	N/A

Abbreviations: AT, anterior tibial muscle; CSA, cross sectional area; EDL, extensor digitorium longus muscle; EM, electron microscopy; SFI, sciatic functional index

concluded positive functional recovery was independent of nerve graft polarity.

In 1988, Ansselin and Davey investigated the influence of graft polarity on regenerating axons sprouting from the proximal stump in a rodent sciatic nerve model ($n = 20$) (Ansselin & Davey, 1988). In both groups, normal myelin sheath was visualized using electron microscopy. In addition, no significant difference was found in mean diameter of myelinated axons or myelin thickness. In the normally oriented graft, regenerating axons sprouted into branches instead of spanning the entire repair zone, which correlated with a decreased distal nerve cross-sectional area. The authors hypothesized the termination of misdirected regenerating axons in the smaller branches consequently resulted in a reduced distal nerve cross-sectional area and overall regeneration. Further, the authors suggested that the orientation of Schwann cells in a reversed graft may minimize misdirected axonal sprouting into the smaller branches. Cross-sectional areas calculated on the distal end of the grafts were consistently less in the normal oriented grafts than the reversed oriented grafts ($P = .002$). The cross-sectional areas of the distal stumps were also found to be significantly smaller in the normal oriented group than the reversed oriented group ($P = .002$) (Ansselin & Davey, 1988).

In a 12-month follow-up study, Ansselin and Davey found small branches in 63% of the normally oriented grafts, which correlated with a smaller cross-sectional diameter than the reversed graft (Ansselin & Davey, 1993). Behavior assessment revealed no significant difference between normal and reverse oriented groups using the sciatic functional index ($P > .05$). However, electrophysiological recordings revealed a significantly higher mean conduction velocity in nerves repaired with a reversed polarity graft than a normal polarity graft ($P < 0.05$). In concordance with these findings, the histological evidence demonstrated the major difference between the normal and reversed grafts was the fate of the branches. At end of three months, while small branches with regenerating axons were found in a majority of normally oriented grafts (5/8), the branches of the reverse oriented grafts did not persist perhaps from decreased misdirected axonal innervation. Histological analysis found the reverse polarity group had a significantly greater number of axons in the distal graft ($P < 0.01$) (Ansselin & Davey, 1993).

In 1992, Sotereanos et al. found nerve graft polarity did not significantly influence motor behavior recovery at 4 months following a 1 cm sciatic nerve lesion using a rodent model ($n = 60$) ($P > 0.1$) (Sotereanos et al., 1992).

Nakatsuka et al. investigated, in 2002, the effect of nerve cable graft polarity in a rabbit model of common peroneal nerve repair ($n = 12$) (Nakatsuka et al., 2002). At 6 months, there were no significant differences between repair groups in motor nerve conduction velocity ($P = 0.65$), muscle weights of the anterior tibialis and extensor digitorum ($P = 0.21$), or axon counts within the graft ($P = 0.94$) or in the distal nerve ($P = 0.96$).

5 | DISCUSSION

When surgically feasible autograft repair is considered the gold standard for repairing gaps in peripheral nerves (Deal et al., 2012). Given the disparity of clinical recommendations amongst peripheral nerve textbooks regarding the need to reverse autografts (Anderl, 1977; O'Brien & Morrison, 1987; Sunderland, 1992; Terzis, 1987), the aim of this systematic review was to provide a critical assessment of the literature regarding autograft polarity and its effects on nerve regeneration and clinical outcomes.

Stromberg et al., Sotereanos et al., Nakatsuka et al., and Sanders and Young all reported no significant differences in histopathological, electrophysiological, or behavioral assessments between the normal and reversed polarity groups (Nakatsuka et al., 2002; Sanders & Young, 1943; Sotereanos et al., 1992; Stromberg et al., 1979). In contrast, Ansselin and Davey did find an effect of nerve branching points on regeneration in an autograft repair. Ansselin and Davey demonstrated a significant decrease in cross-sectional area in the normal orientation group and axon count compared with the reversed polarity group. Furthermore, a greater conduction velocity was reported in the reversed orientation group (Ansselin & Davey, 1988).

Several studies have argued that axons select a path at a branching point in a stochastic manner (English, 2005; Scherer, 1986; Westerfield, 1987; Westerfield & Powell, 1983), while others have stated that regenerating axons follow preferential pathways (Abernethy, Rud, & Thomas, 1992; Grimm, 1971; Kuffler, 1986; Lee & Farel, 1988; Sperry & Arora, 1965; Stephenson, 1979). Isaacman-Beck et al. demonstrated in a zebrafish model that transected axons retain a high degree of target specificity (80%). Their work suggests that Schwann cells neighboring a lesion site induce changes that guide axonal regeneration. Regenerating axons that probe inappropriate trajectories are destabilized, which ensures target-selective regeneration (Isaacman-Beck, Schneider, Franzini-Armstrong, & Granato, 2015). This may explain why no clinical differences are seen between reversed and normal orientation groups, as the majority of axons may retain their target specificity.

Modality preference, that is sensory versus motor pathways, may be equally important for regenerating axons. Brushart et al. argued that regenerating motor axons preferentially re-innervated motor branches after transection. After re-innervation distal to the transection site, specificity is created by selective pruning of the sensory pathways and maintenance of the motor pathways (Brushart, 1988, 1993). These data have clinical significance because motor nerves are commonly repaired using sensory nerve autograft(s). For instance, the sural nerve is commonly used in motor nerve repair (Mackinnon & Dellon, 1988; Terzis, 1987), which as Brushart suggests, may not be ideal for motor axon regeneration. This highlights the need for future studies that directly compare the effects of motor versus sensory graft on functional outcome. Indeed, the studies described above were all performed on normal orientation grafts, highlighting the need for future studies on modality preference in reversed orientation as well.

To date, neither animal nor in vitro studies have been done to directly compare the effects of branching points on nerve regeneration or functional outcome. Specifically, the impact of the presence of multiple branch points on a nerve graft on axonal regeneration is unknown. Such data would be clinically significant because grafts with branching

points, such as the sural nerve, are commonly used in clinical practice (Mackinnon & Dellon, 1988; Terzis, 1987).

Though the sample sizes of the reviewed studies were small, they were powered adequately to detect differences with electrophysiological and histopathological outcomes. The reviewed studies were consistent in showing no behavioral difference between groups, although behavioral outcomes were only directly examined in 2 of the studies.

A theoretical problem with reversing autograft polarity is graft stump diameter mismatch, especially in repairs that require long autografts. This may lead to worse axon regeneration, as not all regenerating axons will be able to travel along a smaller diameter graft. A current clinical solution to this issue is the practice of cable grafting.

A shortcoming of the current literature is its lack of consistency between different animal models (rat versus rabbit) and type of nerve grafts (branch points versus no branch points). The choice of animal model may be particularly important in detecting subtle clinical differences. Given the rat's superior nerve regenerating capacity (Gordon & Borschel, 2016), a large animal model which includes a nerve autograft group with and without branches, would be better suited to answer the question of nerve autograft polarity.

Acellular allografts and emerging bioengineered nerve repair conduits are becoming potential alternatives to autografts (Pfister, Loverde, Kochar, Mackinnon, & Cullen, 2011). Though autologous nerve grafting remains the gold standard, these emerging technologies may offer new options for surgeons who wish to repair a lesion without the cost of donor site morbidity.

6 | CONCLUSION

Despite paucity of data on the topic, none of the studies included have suggested advantages of normal proximal orientation grafts over reversal. A reversed graft may ensure that regenerating nerve fibers are not lost at extraneous branching points within the graft itself. Still, the impact of lost regenerating fibers at branching points may be irrelevant clinically as the current literature has failed to show significant differences in behavior between groups. At this time, we would recommend reversal of autograft polarity with multiple branching points, whereas with a graft with no branch points reversal is not necessary, though future studies are required to further clarify this question.

CONFLICTS OF INTEREST

none.

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