The Incidence of Positive Modifications to Nerve Conduits in Rodent Nerve Repair Models

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Abstract

Background: The nerve conduit is a generally accepted tool to facilitate the repair of short nerve gaps. Limitations in effectiveness have been recognized, and a steady stream of possible conduit improvements has been published in the scientific literature. Analysis of this information, particularly when small animal models have been utilized, seems to indicate that nearly any modification of a nerve conduit improves outcomes in repairs of short gaps over standard nerve conduits. This seems statistically and biologically improbable and suggests a bias in the literature. **Methods:** A standardized systemic review of the scientific literature on rodent model studies assessing conduit modifications was undertaken to determine the incidence of positive or supportive outcomes. **Results:** Modifications were deemed superior in 97.3% of studies when compared with unmodified conduits and deemed equivalent or superior in 52.1% of studies when compared with autograft. **Conclusions:** A seemingly disproportionate number of positive results suggest that the literature on nerve conduit modifications may be skewed. We believe that there is a publication bias in the literature, and this warrants further investigation.

Keywords: nerve conduits, selection bias

Introduction

Biological and nonbiological conduits have become a common tool for overcoming short gaps in peripheral nerve repair. While avoiding the increased surgical time and morbidity associated with harvesting of autograft, they offer an efficient and convenient method for avoiding tension in situations of minimal nerve loss or nerve retraction.^{13,14} There are clinical and experimental data to support this clinical usage; however, results are often unpredictable, particularly with gaps greater than a few centimeters and in larger diameter nerves.^{13,14,27} These limitations have motivated research aimed at evolving and improving current conduit design.

Current conduit designs create a relative protected microenvironment within nerve tubes allowing neurotrophic factors to accumulate and a fibrin clot forms between the nerve ends.^{3,17} This clot, which becomes progressively unstable with increasing lengths, acts as a scaffold to allow Schwann cells and subsequently axons to migrate across the gap.^{3,8,22,25} As researchers move toward the next generation of nerve conduits, common areas of “enhancement” include intraluminal fillers, alterations in conduit wall materials, and the addition of various growth and neurotrophic factors. With such a myriad of potential enhancements, the best

single or even combined approach is difficult to predict.² Small animal nerve regeneration models theoretically provide a research tool that could help unravel this complex question. However, when reviewing the animal literature on nerve conduits to discern what modifications may be of therapeutic benefit, it is often confusing as it seems that all modifications seem to do well. To verify and better understand this observation, we systematically reviewed the available scientific literature with the hypothesis that almost all published rodent conduit repair studies will show positive results, regardless of specific modifying strategy suggesting an underlying bias in the literature.

Methods

We utilized the preferred reporting items for systematic reviews and meta-analyses (PRISMA) method for systematic

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Figure 1. Flow diagram of the screening process.¹⁸

review. The PRISMA method is a standardized approach to systematic reviews and meta-analyses to allow more transparency and reliability. A systematic review is defined as a review of the literature in the context of a specific hypothesis using a systematic and explicit methodology to identify, select, and critically analyze the relevant research. As opposed to a meta-analysis, statistical analysis is not required in a systematic review.¹⁸ Nerve conduit studies utilizing rodent models that compared a modified conduit with a control (which we defined as autogenous nerve graft and/or an empty nerve conduit) were identified using PubMed and Web of Science databases. Using the search term “nerve conduit AND rodent” brought up a total of 416 citations. After removing repeat studies and filtering by “full text” articles in “English,” 361 citations remained. The terms were last used in September 2014 for the purposes of the research project.

The 361 citations were reviewed, and studies meeting inclusion criteria were included. Inclusion criteria required

that the study was performed in rodents, the repair was done on the sciatic nerve or distal branches, and a comparison to a control was made (control could be empty nerve conduit and/or autogenous nerve graft). The conduit did not have to be approved for clinical use by the Food and Drug Administration or equivalent body. All studies that met above criteria were further analyzed, and no exclusion criteria were used (Figure 1).

A total of 75 papers meeting this criteria were reviewed, and following data were extracted: conclusion as per authors, total number of rodents included in study, number of study groups being studied, size of the gap created for the repair, postoperative time at which final analysis was done, specific outcomes measures being analyzed, type of material used for nerve conduit construction, and intraconduit modification (see Table 1).

To simplify the analysis of outcomes measures studied, only outcomes with quantitative measurements (statistically

Table 1. Overview of Data Points Extracted From Studies.

Average No. of rodents	42 (range, 12-195)
Average No. of study groups	3.86 (range, 2-8)
Average gap size	10.9 mm (range, 3-30 mm)
Postoperative time at which final analysis was done	13.6 wk (range, 4-52 wk)
Outcome measures	41
Types of nerve conduit materials	19
Intraconduit modifications	31

analyzed for significance) were compared. Many studies included qualitative analysis of outcomes, which was noted in the final analysis. The principal study measure was the study conclusions and in particular, whether the modified conduits were felt to be superior to empty conduits and/or autogenous nerve grafts. They were classified as either (1) modified nerve conduits versus empty nerve conduits or (2) modified nerve conduit versus autogenous nerve graft. It should be noted that a single study could compare a modified nerve conduit with an empty nerve conduit as well as autogenous nerve graft and would therefore be classified into both categories.

We also investigated whether “follow-up” studies incorporating the modified nerve conduits had been pursued. A secondary literature search identified potential continued scientific evaluation of advanced conduit concepts through search terms “nerve conduit human” and “nerve conduit primate” and yielded 303 and 287 results, respectively.

Results

Overall, 97.3% of the studies comparing modified nerve conduits with empty or saline-filled nerve conduits (67 studies) concluded that the modified nerve conduits were superior. There were only 2 studies that concluded that the modified nerve conduits were equal to control. One study concluded that microspheres filled with nerve growth factor neither improved nor hampered nerve regeneration compared with controls.⁴ The other study found that direct injection of leupeptin (a protease inhibitor) into nerve conduits did not improve nerve regeneration.¹⁰ When comparing modified nerve conduits with autogenous nerve graft (48 studies), a total of 52.1% studies determined that there was either no statistical difference or conduits were better than autograft. Of these, 5 determined that their modifications were “better” based on improved histopathological parameters (though all with equivalent neurophysiological). Of the studies that determined that the modified nerve conduits were inferior to autograft, all of them also made comparisons with an empty/saline-filled nerve conduit, and of these studies, only 1 concluded that their modified nerve conduit did not offer improved results compared with control conduit as referenced above.⁴

A total of 19 different materials were used across all studies to construct nerve conduits with 31 different intraconduit modifications in an effort to improve nerve growth. The most common materials (62 studies) included polysaccharide- or protein-based conduits such as poly (ϵ -caprolactone) (PCA), polylactic acid (PLA), collagen, chitosan, or laminin. Eighteen studies constructed “biological” conduits such as vein/artery, muscle, epineurial grafts, or fibrin-based conduits; 21 studies used silicon-based conduits; and 12 studies used nanofiber technology, which involved electrospinning technique to construct tubes with an internal matrix to certain specifications using silk, polylactic acid, poly(3-hydroxybutyrate-co-3-hydroxyvalerate), and/or polycaprolactone. The most common intraconduit modifications included specific growth factors and/stem cells, either introduced as an injection into conduit or suspended in a matrix (fibrin, hydrogel, or internal nanostructure). All studies that included stem cells and/or growth factors concluded that the modified nerve conduits were all superior to empty conduits and 67% concluded that they were equal or superior (5 studies) to autograft. Some of the more eccentric modifications include the use of stimulating lasers, thyroid hormone, melatonin, and lithium chloride. Interestingly, only melatonin was associated with negative outcome.

We found only 2 “follow-up” studies in our literature review. One study evaluated human umbilical cord mesenchymal stem cell–loaded amniotic membrane for the repair of radial nerve injuries.¹⁶ The other studied human hair keratin scaffold–enhanced median nerve regeneration in nonhuman primates.²⁰

Discussion

We were impressed by how many rodent nerve repair studies on conduit modification seemed to report positive or encouraging results. Even in the absence of scientific rationale, almost all conduit modifications seemed to improve nerve regeneration. This statistically and biologically unlikely scenario has been confirmed by this systemic review in which 97.3% of studies showing that modified nerve conduits are better than empty conduits and 52.1% of studies show modified nerve conduits were better or equal to autogenous nerve grafts.

A worrisome possible explanation for the high numbers of positive conclusions is that this is a result of publication bias. Publication bias is when negative or insignificant results are less likely to be published than positive results. The point of discrimination can be either the investigators (who do not submit their negative results for publication) or the editors (who do not publish submitted negative results). Most of the literature on publication bias in the medical field is mixed, but, in general, it does seem to lead to a disproportionate publishing of positive results.

A study by Cochrane Database of Systematic Reviews analyzed the publication of abstracts previously presented

at scientific meetings.²³ It was found that eventual publications were associated with positive results (relative risk 1.17 [95% confidence interval, 1.02-1.35]; $P = .03$) and the reporting of significant results (relative risk 1.30 [95% confidence interval, 1.14-1.47]; $P = .00004$).²³ These observations were mirrored in analyses of specific medical specialties of emergency medicine, cardiovascular, and pediatrics.^{1,15,21} In terms of orthopedic surgery, a review of abstracts presented at the American Academy of Orthopedic meeting showed research with positive outcomes and significance being associated with eventual publication.⁹

Okike et al attempted to quantify the effects of publication bias in the orthopedic literature by analyzing the factors associated with publication in the *Journal of Bone & Joint Surgery (JBJS)*. After viewing manuscripts submitted to *JBJS* for publication in an 18-month period, they found that 23% of studies with insignificant and negative results were accepted for publication versus 21.3% of studies with positive results suggesting no publication bias by the editors. However, with remarkably less insignificant or negative results submitted in the first place, they concluded that the bias may be at the investigator level.¹⁹ Dickersin et al came to similar conclusions based on their review of factors associated with the publication of research findings at Johns Hopkins Health Institute. They determined an odds ratio of 2.54 (95% confidence interval, 1.63-3.94) associated with significant result reporting. Only 6 of the 124 studies with insignificant results were reportedly published.⁷

The overrepresentation of positive results can also skew meta-analyses in favor of positive outcomes. Several authors have suggested accounting for publication bias and even developed methods to decrease its effect on meta-analyses. Vavken et al found that 12% of the meta-analyses were affected by publication bias. The fact that the affected studies overestimated effect size is not surprising, as the studies missing were negative, that is, provided evidence for “no effect.” Although Vavken et al did not find a statistical difference between the bias-adjusted and unadjusted results, publications from other fields of medicine have found that this difference can be significant enough to affect final conclusions.²⁴

There may be other factors contributing to the overwhelming positive results, and other researchers have also noted difficulties in meaningful interpretation of the scientific literature.⁵ Variation in evaluation techniques has long been recognized as an obstacle in comparing different nerve repair strategies in rodent models.^{6,26} We found 41 different types of objective outcome measurement taken at varying intervals from 4 to 52 weeks over 75 papers. It is possible with multiple techniques being used for outcome analysis that there is a bias toward those that seem to best support the hypothesis.¹¹ Irintchev noted that the assessment of functional recovery of the sciatic nerve in rodents is challenging

due to its functional and anatomic complexity.¹² Woods et al evaluated 6 outcome measures in rodent models and noted that though they may accurately represent specific qualities of nerve recovery, they can be used to come to erroneous conclusion on overall nerve recovery.²⁶ In addition, though we were only able to identify two “follow-up” studies, this may be explained by funding issues, difficulties with institutional review board approval, or transient enthusiasm for a given type of modification.

Only 75 of a possible 361 studies were included in final analysis. Most citations were excluded because they did not pertain to nerve repairs done in rats. The second and third most common reasons for exclusion were biomaterial studies focusing on the functionality of the tubes without in vivo analysis and studies that did not include adequate control groups. Some studies used conduits as a tool to study growth factors or the roles of different cells in supporting regeneration rather than as specific conduit “modifications” and would therefore not be included in this systemic analysis. Broader terminology such as *nerve tubes* (instead of *conduits*) may have yielded higher numbers of studies, as well. However, the chosen search terms seemed to identify enough studies to effectively evaluate our hypothesis. Finally, it deserves emphasis that we did not judge the quality or scientific validity of specific study outcomes, only the incidence of positive conclusions.

Although this study does not analyze the factors related to the remarkably high positive results reported in a disproportionate number of in vivo rodent studies assessing nerve conduit modifications, our goal was to confirm our impression that animal conduit studies were skewed. Though agreement regarding the standardization of outcomes measures would be quite difficult across the many disciplines studying nerve regeneration, this would certainly be a meaningful start in improving the quality of the current literature. Furthermore, the recognition of what does appear to be a publication bias as well as the associated detrimental consequences may lead to a greater willingness to submit negative results.

Ethical Approval

This study was approved by our institutional review board.

Statement of Human and Animal Rights

This article does not present original research and, therefore, does not include a statement of human or animal rights.

Statement of Informed Consent

No patient information is included in this article.

Declaration of Conflicting Interests

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