Published in final edited form as:

Neurosurgery. 2009 October ; 65(4 Suppl): A52–A54. doi:10.1227/01.NEU.0000335655.66073.27.

A Tribute to Dr. David Kline: A New Approach to an Old Peripheral Nerve Problem—Splicing Instead of Regenerating Disrupted Axons

Wesley Chang, Ph.D. and David Sretevan, M.D., Ph.D

Departments of Ophthalmology and Physiology, Neuroscience Program and Bioengineering Program, University of California, San Francisco, San Francisco, California

Michel Kliot, M.D.

Department of Neurosurgery, School of Medicine, University of Washington, and Puget Sound Veterans Administration Health Care Center, Seattle, Washington

Abstract

OBJECTIVE—Our goal is to develop a novel method to repair damaged axons. This method relies on acutely restoring axonal continuity rather than the traditional approach of promoting axonal regeneration.

METHODS—Micro- and nanoechnological methods, in combination with focal application of electrical fields, are applied to individual and groups of axons both in vitro and in vivo.

RESULTS—Application of these techniques has permitted micromanipulation of axons at the cellular level and fusion of axonal membranes.

CONCLUSION—Although a great deal more work is necessary, our findings suggest that it may one day be possible to repair acutely disrupted axons by splicing their membranes back together.

Keywords

Acute repair of axons; Axons; Fusion; Nanotechnology; Repair; Splicing

Much attention and effort has been devoted to devising methods of promoting axonal regeneration over the past century and longer. It has certainly been appreciated that the regeneration of axons can and does occur over long distances in the adult mammalian peripheral nervous system and, to a much more limited extent, if at all, in the adult mammalian central nervous system (5). Refinements in experimental techniques and methodology have also shown that even when regeneration of peripheral nerves occurs, the number of axons finally reaching their original target structures is only a fraction of what it was originally, and the accuracy of reinnervation is quite degraded (3). Therefore, although axonal regeneration with recovery of function can and does occur in the peripheral nervous system of animals and patients, new methods of increasing both the quantity and specificity of regrowing axons are needed and are being actively pursued.

Copyright © 2009 by the Congress of Neurological Surgeons

Reprint requests: Michel Kliot, M.D., Department of Neurosurgery, Puget Sound VA Health Care Center, 1660 S. Columbian Way, Seattle, WA 98108. kliot@u.washington.edu.

Disclosure David Sretevan, M.D., Ph.D., discloses financial interest in Mynosys Inc., a company developing microdevices for medical use. The other authors have no personal financial or institutional interest in any of the drugs, materials, or devices described in this article.

The earliest report of the idea of suture repairing a damaged peripheral nerve was written by Paul von Aegina in the 7th century (6). Ironically, it was not until a millennium later that the surgical repair of a severely damaged peripheral nerve was shown to have clinical benefit (6). Only recently have advances in surgical technique been made that significantly improve clinical outcome. One such important advance, made by Dr. David Kline, was the intraoperative application of electrophysiological techniques to determine whether or not a damaged nerve segment should be resected and repaired (4). Dr. Kline and his colleagues showed that intraoperative nerve conduction studies could distinguish a neuroma in continuity with regenerating axons giving rise to a recordable nerve conduction response (axonotmetic grade of injury) from one without conducting axons and a recordable response (neurotmetic grade of injury) several months after a traumatic nerve injury. This distinction is critically important, because the axonotmetic injury does not require a direct nerve repair, whereas the neurotmetic injury requires surgical resection of the intraneural scar tissue impeding regeneration followed by anastomosis of the nerve with or without a graft, depending on the length of the gap. Such advances in peripheral nerve surgery have brought us to the point at which further progress that relies on recovery through axonal regeneration is likely to depend more on biological discoveries than technical innovations.

With the advent of micro- and nanotechnologies, an alternate and novel strategy has recently been proposed that does not rely on the regeneration of axons following damage sufficient to cause loss of axonal continuity (i.e., at least an axonotmetic grade of injury) (1, 2, 8). Instead, this strategy is based on re-establishing axonal continuity soon after the injury and before distal Wallerian degeneration of axons occurs. It relies on acutely re-approximating the proximal and distal stumps of axons and splicing their membranes back together. This idea is analogous to splicing electrical wires back together instead of laying down an entirely new electrical cable. With the help of newly developed microscale surgical tools, the peripheral nerve surgeon would reach down to the subcellular level and manipulate the “wires” of the nerve. The obvious advantage of such an approach is that, if successful, the original pathways are restored and therefore, at least in theory, recovery of function could approach normal baseline values if all or most axons could be biologically spliced back together. The other advantage is the speed of recovery without having to rely on regeneration that occurs at a maximum of 1 mm/d.

Of course, this ambitious goal relies on a surgical “tool belt” that is not yet available. However, advances in micro- and nanotechnology are making it possible to both visualize and manipulate biological structures at the cellular and subcellular levels. In addition, experimental methodologies already exist for fusing together cellular membranes. Therefore, we think that the time is ripe to combine these old and new technologies to take surgery to the cellular and subcellular levels and consider splicing disrupted axons back together instead of and/or in combination with ongoing efforts to promote and enhance axonal regeneration.

We envision that axonal splicing would require at least 3 major steps: 1) trimming the cut ends of axons, 2) moving axons so that their proximal and distal cut ends can be placed in close approximation, and 3) fusing the axonal membranes so as to re-establish both anatomic and functional continuity of the axon (Fig. 1). All of these steps would need to be performed soon after an injury with disruption of axonal continuity to avoid having to deal with the consequences of distal Wallerian degeneration of axons. Combining the expertise of a multidisciplinary group, which includes neurobiologists, with the necessary anatomic and electrophysiological tools, engineers with the capacity to apply appropriate micro- and nanotechnologies, and peripheral nerve surgeons with clinical perspective and experience, we have succeeded in demonstrating proof of concept for each of these steps in vitro and, in some cases, in vivo. Figure 1, A and B, demonstrate the use of a microknife to cut groups or

individual axons both in vitro and in vivo. Figure 1, C and D, demonstrate the capacity using changing electrical fields (dielectrophoresis) to move an axon in vitro without requiring physical contact. Figure 1, E and F, demonstrate the capacity to disrupt and then fuse axonal membranes using electrofusion in vitro.

In order to bring this technology in to the clinical arena, many additional steps are necessary. It will be necessary to demonstrate each of these steps on single axons and then demonstrate that the spliced axons survive and are capable of function. In order to make this novel approach of repairing axons practical, it will be necessary to repair axons quickly, which will likely require that many axons be repaired simultaneously in an automated fashion using computer robotic systems that the surgeon will control and oversee (7). To do so, it will also be necessary to create a user-friendly interface that is compatible with the operating room environment. It will also be necessary to operate on these severe nerve injuries within a short amount of time, before the distal axons degenerate, which may not always be possible. New methods of keeping the distal axons viable for longer periods of time would make it possible to operate on a greater number of severe injuries. Although many challenges remain, we think that the goals and their potential clinical benefits are sufficiently important to warrant our continuing with efforts to further develop and eventually make this approach and technology a clinical reality in the not too distant future (1, 2, 8). If we succeed, our efforts will be a tribute to Dr. Kline, who has made so many important contributions to the field of peripheral nerve surgery, inspired so many peripheral nerve surgeons, and provided the essential foundation for these future advances.

REFERENCES

1. Chang WC, Hawkes EA, Kliot M, Sretavan DW. In vivo use of a nanoknife for axon microsurgery. *Neurosurgery*. 2007; 61:683–692. [PubMed: 17986929]
2. Chang WC, Sretavan DW. Microtechnology in medicine: The emergence of surgical microdevices. *Clin Neurosurg*. 2007; 54:137–147. [PubMed: 18504911]
3. Fu SY, Gordon T. The cellular and molecular basis of peripheral nerve regeneration. *Mol Neurobiol*. 1997; 14:67–116. [PubMed: 9170101]
4. Kline DG, Happel LT. Penfield Lecture. A quarter century's experience with intraoperative nerve action potential recording. *Can J Neurol Sci*. 1993; 20:3–10. [PubMed: 8467425]
5. Lazar, D.; Fitch, M.; Silver, J.; Kliot, M. Injury and recovery in the peripheral and central nervous system. In: Winn, HR.; Youmans, JR., editors. *Youmans Neurological Surgery*. ed 5. W.B. Saunders; Philadelphia: 2003. p. 195-213.
6. Little KM, Zomorodi AR, Selznick LA, Friedman AH. An eclectic history of peripheral nerve surgery. *Neurosurg Clin N Am*. 2004; 15:109–123. [PubMed: 15177311]
7. Nathoo N, Cavaşoğlu MC, Vogelbaum MA, Barnett GH. In touch with robotics: Neurosurgery for the future. *Neurosurgery*. 2005; 56:421–433. [PubMed: 15730567]
8. Sretavan DW, Chang W, Hawkes E, Keller C, Kliot M. Microscale surgery on single axons. *Neurosurgery*. 2005; 57:635–646. [PubMed: 16239875]

FIGURE 1.

In the direct repair scheme recently proposed, novel microsurgical instruments directly manipulate individual axons in an injured nerve. A microcutting tool cuts each axon to remove damaged segments, leaving a discrete, resealing stump. Electrostatic forces are then used to align and juxtapose individual axonal segments in preparation for rejoining. Electrofusion is then applied to the juxtaposed axons, splicing the segments together and leading to reconstitution of a functional axon. **A**, schematic illustrating this technique. **B**, a microcutting tool is positioned over a single axon. **BC**, the axon has been cut (arrow). The tool is able to deliver neat and precise cuts to individual axons, leaving a discrete end that can reseal. Following the cutting, intact axonal segments are aligned and juxtaposed with each other ahead of the splicing step. **D** and **E**, the stretching and aligning of axons between electrodes due to the application of dielectrophoretic force (arrows). Using electrofusion, the juxtaposed axons are joined together to form a single intact unit. **F** and **G**, the fusion of 2 axons allows the fluorescent protein in 1 axon to diffuse into its previously fluorescent-free partner (double arrows). Retention of the fluorescence indicates that membrane continuity is maintained.