# The Effect of Short Nerve Grafts in Series on Axonal Regeneration Across Isografts or Acellular Nerve Allografts

Ying Yan, MD, PhD,* Matthew D. Wood, PhD,* Daniel A. Hunter,* Xueping Ee, MD,* Susan E. Mackinnon, MD,* Amy M. Moore, MD*

**Purpose** To evaluate the regenerative effect of the additional suture line when using either isografts (ISOs) or acellular nerve allografts (ANAs) placed end-to-end to span a short gap in a rat model.

**Methods** Rat sciatic nerves were transected and repaired with 2-cm nerve grafts (ISO or ANA). The grafts were 2 cm in length or a 1-cm segment was connected end-to-end to a 1-cm segment to yield a 2-cm length. At 8 weeks, extensor digitorum longus (EDL) muscle force and mass were measured. Nerves were harvested for histomorphometry. In a separate parallel study, the nerves were harvested 2 weeks following graft implantation to assess gene expression changes.

**Results** All grafts demonstrated regeneration across the 2-cm segment(s). The additional suture line did not result in statistical differences in the number of myelinated nerve fibers that reached the distal nerve. However, when the graft types were compared, there was a significant decrease in nerve fibers in the ANA groups. The EDL muscle mass was significantly greater by using nerve ISOs compared with ANAs, regardless of an additional suture line, but there were no statistical differences noted in EDL muscle force. Gene expression analysis did not differ owing to an additional suture line.

**Conclusions** Minimal axonal loss and no functional deficits were identified with an additional suture line in this rodent short nerve gap model.

**Clinical relevance** Placing nerve grafts in series is a viable option for treating short nerve gaps; however, the use of autografts remains preferable over the use of ANAs. *(J Hand Surg Am. 2016;41(6):e113–e121. Copyright © 2016 by the American Society for Surgery of the Hand. All rights reserved.)*

**Key words** Coaptation site, nerve regeneration, peripheral nerve, processed nerve allograft, suture line.

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*From the *Division of Plastic and Reconstructive Surgery, Washington University School of Medicine, St. Louis, MO.*

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**Corresponding author:** Amy M. Moore, MD, Division of Plastic and Reconstructive Surgery, Washington University School of Medicine, 660 South Euclid, Campus Box 8238, St. Louis, MO. 63110; e-mail: mooream@wustl.edu.

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Nerve grafting is performed when a direct end-to-end coaptation is not possible owing to a segmental nerve injury or tension. Nerve grafting inherently predisposes regenerating axons to an additional coaptation site increasing the risk of axonal loss from scarring.[1,2] Sutures activate a local inflammatory response, which, if large enough, can generate considerable fibrosis hindering the progression of regenerating axons.[3] The primary coaptation site linking the distal and proximal nerve is associated

with slowed or staggered axonal regeneration.[4] Thus, it follows that additional coaptation sites may generate similar effects.

The impact of an additional suture line on axonal regeneration through nerve grafts is not clearly understood. This information holds clinical value in the event of limited donor nerve supply such as that seen with major injuries involving multiple limbs with large segmental nerve defects. In these devastating cases, the end-to-end linking of multiple nerve grafts in series (autograft and/or acellular nerve allografts [ANAs]) to repair long nerve gaps have been described and clinically referred to as "daisy-chaining."[5]

The clinical use of ANAs has increased markedly since the U.S. Food and Drug Administration's approval of the Avance Nerve Graft (AxoGen, Inc., Alachua, FL) was obtained.[6] The decellularized nerve allografts offer an off-the-shelf alternative to autografting and avoid donor site morbidity and prolonged operative time. The potential for using multiple decellularized nerve grafts placed in series is an attractive option, especially in the severely traumatized patient. Our study sought to determine how the additional suture lines affect axonal regeneration across short nerve autografts and acellular nerve allografts. In this study, short nerve grafts were used to avoid additional confounding issues affecting axonal regeneration, such as complete regenerative failure associated with long ANAs.[7] The present study evaluated axonal regeneration and functional recovery across isografts (ISOs; animal model equivalent of an autograft[8,9]) and ANAs repaired using a whole, unmodified graft or a graft of comparable length with an additional suture line. To determine how the additional suture line exerted its influence, gene expression products related to nerve regeneration were measured in the grafts.

## MATERIALS AND METHODS

### Animals and experimental design

The sciatic nerves of adult male Lewis rats (250 g; Charles River Laboratories, Wilmington, MA) were transected and then immediately repaired with 2-cm nerve grafts. These grafts were either left unmodified (whole) or cut into equal 1-cm pieces and sutured end-to-end, thus generating an equivalent 2-cm length. A 2-cm nerve gap was chosen to eliminate the confounding effects of graft length and to focus on the effect of the additional suture line on regeneration. Long nerve grafts (autograft and ANA) have been associated with a significant decline in axonal regeneration in comparison with short nerve grafts.[7] Further, a 2-cm graft can be obtained from the sciatic nerve with consistent diameter and no additional branch points, eliminating confounders such as axonal loss and graft size.

In study A, 32 rats were divided into 4 groups (n = 8 per group) consisting of a whole ISO, an ISO with an additional suture line (ISO in-series), a whole ANA (ANA), or an ANA with an additional suture line (ANA in-series). At 8 weeks after surgery, extensor digitorum longus (EDL) muscle force testing was performed, and EDL muscle mass was measured. The sciatic nerves were also harvested for histomorphometric analysis (Table 1). Eight weeks was chosen based on previous studies that suggest this time point maximizes the sensitivity to measure differences between groups receiving 2-cm nerve grafts.[10,11]

In a parallel study B, 16 rats were randomized to the same 4 groups (n = 4 per group) and underwent similar procedures as in study A. These rats were used to assess gene expression within the graft area affected by the suture line (or in the midgraft of the whole grafts). At 2 weeks after surgery, gene expression was measured using quantitative reverse-transcriptase–polymerase chain reaction (qRT-PCR; Table 1).

For all groups, Lewis male rats served as the experimental animals and donor animals for ISO groups. In ANA graft groups, Sprague-Dawley rats (250 g; Charles River Laboratories, Wilmington, MA) were used as donor nerve for processing to generate ANAs. The Sprague-Dawley (SD) (*RT-1*$^{b}$ major histocompatibility complex [MHC]) rat strain is MHC-incompatible with Lewis (*RT-1*$^{1}$ MHC) and were used as allograft donors. Lewis rats are a well-accepted rat strain used to derive ISOs because they are inbred leading to syngeneic donors. Sciatic nerve allografts harvested from donor rats were chemically processed and decellularized using a series of detergents as previously described.[7,12,13]

Surgical procedures and perioperative care measures were conducted in compliance with the Institutional Animal Studies Committee and National Institutes of Health guidelines. All animals were housed in a central animal care facility and provided with food (PicoLab rodent diet 20; Purina Mills Nutrition International, St. Louis, MO) and water *ad libitum*.

### Surgical procedures

Surgical procedures were performed under aseptic conditions and with the aid of an operating microscope under magnifications of 10-25X.[7] A single surgeon (Y.Y.) performed all operations. Anesthesia was provided by subcutaneous delivery of ketamine (75 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) and dexmedetomidine (0.5 mg/kg; Pfizer Animal