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CASE REPORT
Peripheral Nerve

Histopathological Confirmation of Axonal Sprouting in Regenerative Peripheral Nerve Interface

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Summary: Symptomatic neuroma represents a debilitating complication after major limb amputation. The regenerative peripheral nerve interface (RPNI) has emerged as a reproducible and practical surgery aimed at mitigating the formation of painful neuroma. Although previous animal studies revealed axonal sprouting, elongation, and synaptogenesis of proximal nerve stump within the muscle graft in RPNI, there is a lack of reports confirming these physiological reactions at the histopathological level in human samples. This report presents a case of below-knee amputation with RPNI due to foot gangrene resulting from polyarteritis nodosa. Subsequently, an above-knee amputation was necessitated due to the exacerbation of polyarteritis nodosa, providing the opportunity for histopathological examination of the RPNI site. The examination revealed sprouting, elongation, and existence of neuromuscular junction of the tibial nerve within the grafted muscle. To the best of our knowledge, this is the first report demonstrating axonal sprouting, elongation, and possibility of synaptogenesis of the nerve stump within the grafted muscle in a human sample. *(Plast Reconstr Surg Glob Open 2024; 12:e5878; doi: 10.1097/GOX.0000000000005878; Published online 6 June 2024.)*

A major limb amputation often results in painful and disabling sensory experiences, hindering prosthesis use and diminishing overall quality of life. Residual limb pain after amputations is commonly attributed to neuroma formation, occurring in 12%–50% of cases.[1]

The regenerative peripheral nerve interface (RPNI) has recently emerged as a reproducible surgical procedure aimed at reducing painful neuroma formation in the clinical settings.[1,2] It entails implanting a proximal nerve stump into a free skeletal muscle graft, originally intended to transduce and amplify neural signals for controlling a neuroprosthetic limb.[3] The use of free muscle grafts during RPNI surgery offers an ample supply of denervated muscle targets, facilitating the regeneration of axons sprouting from the proximal nerve stump.[3] In rat experiments, RPNI has demonstrated the capacity to generate compound muscle action potentials as early as one month after implantation, achieved by embedding electrodes within the muscle during surgery to measure electrical potentials postoperatively.[3] In human upper limb amputation cases, a study investigated the motor potentials of free muscle grafts that underwent RPNI with surgically inserted electrodes. The study showed that these motor potentials could effectively serve as a control signal for operating a prosthetic hand.[4] These findings indicate the concurrent formation of new neuromuscular junctions (synaptogenesis) within the muscle graft. However, to date, only animal studies have confirmed the occurrence of axonal sprouting, elongation, and synaptogenesis within muscle grafts on RPNI in histopathological examination.[3,5,6]

In this report, we present a case where the histopathological examination of the grafted muscle with primary RPNI became feasible due to an incidental additional amputation. This unique circumstance confirmed the synaptogenesis after RPNI in a human sample.

CASE REPORT

A 61-year-old woman presented with progressing bilateral lower limb numbness and paralysis over 3 months.

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The patient was diagnosed with polyarteritis nodosa by bilateral sural nerve and peroneus brevis muscle biopsies. Four months later, she experienced necrosis in both feet. Despite steroid pulse and intravenous cyclophosphamide therapy, the necrosis progressed, leading to bilateral below-knee amputations after 6 months from symptom onset.

RPNIs were performed along with amputations on both limbs following the technique developed by Kubiak et al.[3] The proximal tibial and deep peroneal nerve ends were encased with small free gastrocnemius muscle grafts to construct RPNIs. The tibial nerve was split into three fascicles to create three separate RPNIs, whereas the deep peroneal nerve was used without splitting for a single RPNI. Free muscle grafts (size: approximately $3 \times 1 \times 1.5$ cm) were harvested, selecting a healthy and normal portion from the proximal end of the amputated limb's gastrocnemius muscle. All suturing was performed with 6-0 nonabsorbable monofilament. The nerve end was initially secured to the center of the muscle graft using two or three epineural-to-epimysial stitches. Subsequently, the muscle grafts were wrapped and secured around the nerve with additional epimysial stitches, and two extra stitches were placed for support from the proximal edge of the muscle grafts to the adjacent epineurium (Fig. 1). After constructing all RPNIs, the surgical site was closed.

After the surgery, the patient had wound dehiscence in the left limb, effectively managed with minor debridement at the outpatient level, resulting in complete healing 6 months later with no neuropathic or phantom pain in both limbs. However, after 1.5 years from the initial surgery, a recurrence of wound necrosis at the left lower limb surgical site occurred during prosthetic adjustments, necessitating a higher-level secondary amputation above the knee due to exacerbated polyarteritis nodosa. Specimens for histopathological examination were extracted from the portion of the left lower limb amputated during the second surgery, where RPNIs were initially performed. The wound healed uneventfully, and rehabilitation using lower limb prostheses began 6 months after the secondary operation. One-and-a-half years after the secondary amputation, the patient could walk using both lower limb prostheses with the assistance of a pickup walker.

The histopathological examination revealed sprouting, elongation, and existence of neuromuscular junctions of the tibial nerve in the free gastrocnemius muscle graft. Hematoxylin–eosin and neurofilament staining demonstrated the proximal stump of the tibial nerve extended into the grafted muscle (Figs. 2 and 3). An immunofluorescent image using neurofilament and acetylcholinesterase double staining revealed axonal sprouting and elongation of the proximal stump of the tibial nerve within the grafted muscle, confirming the existence of neuromuscular junctions (Fig. 4).

DISCUSSION

Symptomatic neuromas pose a significant challenge in managing postoperative pain after limb amputation. Various surgical interventions, including the implantation of the proximal nerve stump into the muscles or veins[7] and covering the nerve stump with acellular nerve allografts[8] or tissue-engineered caps,[9] have been explored to prevent traumatic neuroma formation. However, consensus on the optimal technique for long-term benefits remains elusive. RPNI has emerged as an innovative strategy for neuroma management.[10] Animal studies have demonstrated the regeneration, revascularization, reinnervation, and overall efficacy of RPNI for preventing and treating neuromas.[3,5,6]

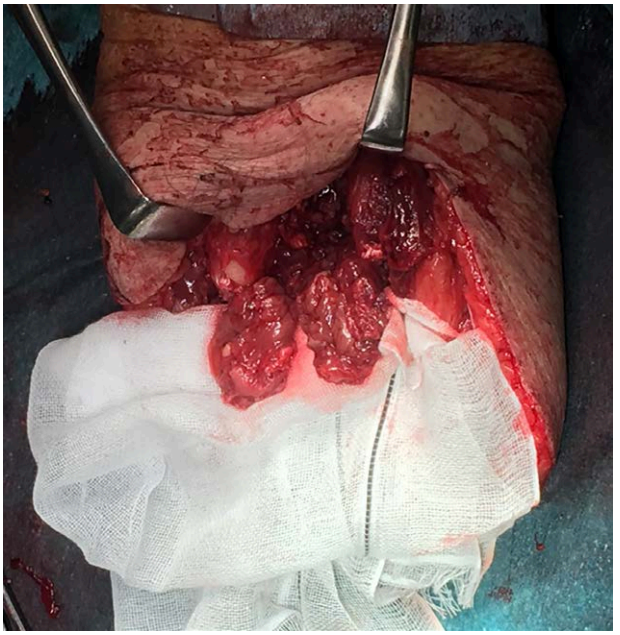

Fig. 1. Intraoperative photograph: split tibial nerve, wrapped with a free muscle graft through the RPNI reconstruction.

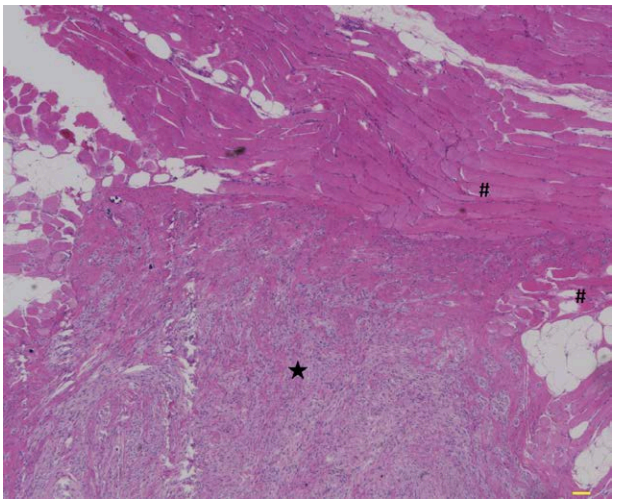

Fig. 2. Histopathological findings: Hematoxylin–eosin staining showing that the proximal nerve stump of the tibial nerve (★) extended into the free muscle graft (#). Scale bars: 100 μm.

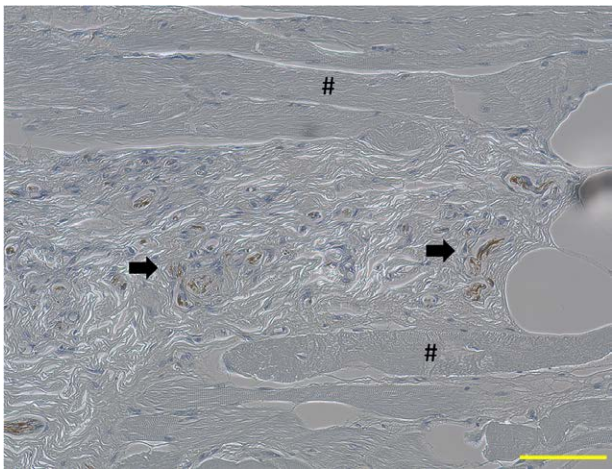

Fig. 3. Histopathological findings: Neurofilament staining revealing that axons (arrows) extended into free muscle graft (#). Scale bars: 100 μm.

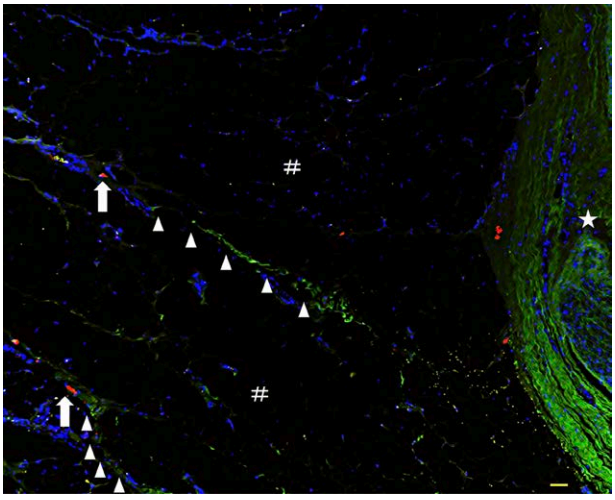

Fig. 4. Histopathological findings: neurofilament and acetylcholinesterase double immunofluorescent image demonstrating axonal sprouting and elongation (▲) of the proximal nerve stump of the tibial nerve (★) to the grafted muscle (#), confirming the existence of neuromuscular junction (arrows). Neurofilament antibody is represented in green, acetylcholinesterase antibody in red, and nuclei in blue using DAPI staining. Scale bars, 100 μm.

Despite these advancements, studies demonstrating the histopathological findings of an implanted nerve stump post RPNI in human subjects are lacking. Our report fills this crucial gap, presenting the first evidence of synaptogenesis potential at the RPNI site using a human sample. Ethical constraints typically hinder acquiring human samples from RPNI recipients in clinical settings. Our unique case, necessitating additional amputation due to disease exacerbation, provided a rare opportunity to analyze an implanted nerve stump post RPNI. One limitation is that the assessment of RPNI success was based solely on histopathological examination and absence of neuropathic pain or phantom pain. Although alternative methods like CMAP or ultrasound, used to detect motor potential and observe muscle contraction, were possible, practical constraints in our specific case prevented their use.[4]

In conclusion, our study represents a significant stride in translating RPNI efficacy from animal studies to human application, reaffirming RPNI as a viable neuroma management strategy in limb amputation. Despite the rarity of multilevel amputation cases, we plan to thoroughly evaluate future instances, regardless of prior RPNI, to validate our findings.

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DISCLOSURES

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