Journal of Translational Medicine

https://doi.org/10.1186/s12967-026-08453-y

Article in Press

Regenerative Peripheral Nerve Interface (RPNI) and vascularized Denervated Muscle Targets (VDMT): a preclinical rabbit model as a translational feasibility and methodological platform

Received: 29 January 2026

Accepted: 7 June 2026

Published online: 24 June 2026

Cite this article as: González-Prieto J., Arenillas M., Sanz Barbero E. *et al.* Regenerative Peripheral Nerve Interface (RPNI) and vascularized Denervated Muscle Targets (VDMT): a preclinical rabbit model as a translational feasibility and methodological platform. *J Transl Med* (2026). https://doi.org/10.1186/s12967-026-08453-y

Jorge González-Prieto, Mario Arenillas, Elisa Sanz Barbero, Ana Gutiérrez Pecharromán, José Daniel Muñoz, Eduardo Alonso, María del Carmen Morillo, Lara Cristóbal, Romano Giannetti & Andrés A. Maldonado

Regenerative Peripheral Nerve Interface (RPNI) and Vascularized Denervated Muscle Targets (VDMT): a preclinical rabbit model as a translational feasibility and methodological platform.

Jorge González-Prieto, MD[1,2]; Mario Arenillas, PhD[3]; Elisa Sanz Barbero, MD[4]; Ana Gutiérrez Pecharromán, MD[5]; José Daniel Muñoz, PhD [6]; Eduardo Alonso, PhD[6]; María del Carmen Morillo, PhD[7]; Lara Cristóbal, MD, PhD[1,2]; Romano Giannetti, PhD[+6], Andrés A. Maldonado, MD, PhD[+1,2].

1. Department of Plastic Surgery, Peripheral Nerve Unit, Hospital Universitario de Getafe, Madrid, Spain.

2. Department of Medicine; Faculty of Medicine, Health and Sports; Universidad Europea de Madrid, Spain.

3. Department of Animal Medicine and Surgery, Veterinary Faculty, Universidad Complutense de Madrid, Madrid, Spain.

4. Department of Clinical Neurophysiology, Peripheral Nerve Unit, Hospital Universitario de Getafe, Madrid, Spain.

5. Department of Pathological Anatomy, Peripheral Nerve Unit, Hospital Universitario de Getafe, Madrid, Spain.

6. Institute for Research in Technology, ICAI School of Engineering, Comillas Pontifical University, Madrid, Spain.

7. Department of Topographic Engineering and Cartography, Polytechnic University of Madrid, Spain.

+These authors shared senior authorship in this work.

Corresponding author:

Andrés A. Maldonado

Department of Plastic Surgery

University Hospital of Getafe Madrid

Carretera de Toledo Km 12.5,

28905-Getafe, Madrid (Spain).

mail@andresmaldonado.es

Financial disclosure: This study has been funded by Instituto de Salud Carlos III (ISCIII) through the project PI22/00862 and co-funded by the European Union. The funders had no role in the study design, data collection or analysis, decision to publish, or manuscript preparation.

Conflicts of Interest: The authors declare that there are no relevant conflicts of interest.

Presented at (if applicable):

This study has not been presented at any meeting or conference.

Short Running Head:

RPNI and VDMT in Rabbit Model

Keywords: Regenerative Peripheral Nerve Interfaces, Vascularized Denervated Muscle Targets, prosthetic control, preclinical model, neuromuscular interfaces, rabbit model.

Abstract

Background: Regenerative Peripheral Nerve Interfaces (RPNIs) and Vascularized Denervated Muscle Targets (VDMTs) are emerging strategies for nerve regeneration and myoelectric prosthesis control. Most preclinical studies have been conducted in rodents, limiting the evaluation of implantable electromyographic devices due to anatomical and scale-related constraints. This study aimed to develop and evaluate the first RPNI and VDMT models in rabbits as a translational platform for implantable neuromuscular interfaces.

Methods: A total of 8 rabbits (4 per group) were randomly assigned to RPNI (n = 4) and VDMT (n = 4) groups and evaluated at an 8-week follow-up. RPNIs were created using biceps femoris muscle grafts attached to the peroneal nerve. VDMTs were constructed using denervated, vascularized gastrocnemius muscle flaps sutured to a peroneal motor branch. The contralateral leg served as a denervated control. Electrophysiological and histological analyses were performed 8 weeks postoperatively. Between-group comparisons were conducted using exact Mann–Whitney U and Fisher's exact tests.

Results: VDMTs showed a trend toward better macroscopic vascularization and integration under the present experimental conditions. Electromyographic activity was detected in 3 of 4 VDMTs, whereas no signal was observed in RPNIs. Histological analysis demonstrated more favorable muscle tissue preservation in VDMTs, with no detectable necrosis (0% necrotic area in all specimens), while RPNIs exhibited substantial necrosis (20%–95% of the analyzed muscle area), which was significantly greater than in VDMTs ($p = 0.0286$). Neuroma formation occurred in all RPNIs and in 2 of 4 VDMTs.

Conclusions: This study presents a feasible rabbit model for the preclinical evaluation of RPNI and VDMT constructs. Under the present experimental conditions, VDMTs were associated with more favorable tissue preservation and more frequent signal detection under

the present experimental conditions. These findings support the feasibility and translational potential of the rabbit as a methodological platform for future neuromuscular interface research. Further studies with optimized surgical protocols and larger cohorts are required to confirm these findings.

Introduction

Human–machine interfaces play a fundamental role in the restoration of motor function after peripheral and central nervous system injuries, enabling individuals with chronic tetraplegia to regain limb movement [1]. However, developing advanced and reliable interfaces for precise control of bionic prostheses remains a challenge, requiring preclinical evaluation of biocompatibility and functionality [2,3].

Regenerative Peripheral Nerve Interfaces (RPNIs), introduced by the Michigan group [4–7], involve grafting a small muscle segment onto a nerve stump of a residual limb [4–10]. Peripheral axons sprout and reinnervate the muscle graft within 2–4 months, forming a "bioelectrode" that transmits signals from the nervous system to external prostheses [4,6,7,11–15].

The RPNI technique has been widely studied in rodents, showing promising histological and neurophysiological results, including muscular neoangiogenesis, axonal regeneration, and minimal fibrosis [4,5,7,8,12,14,16–25]. Although applied in rats [4,5,8,10,12,14,16–41] and macaques [25,42,43], no single species fully replicates human physiology, supporting the need for complementary animal models [44]. While primates provide valuable insights [45,46], ethical and financial concerns limit their use [46].

Vascularized Denervated Muscle Targets (VDMTs) have emerged as an alternative strategy for neuroma management and potential myoelectric interfacing [7,47–51]. Unlike RPNIs, VDMTs (also described as pedicled vascularized RPNIs) use a vascularized, denervated muscle flap wrapped around the nerve stump, improving viability and eliminating size constraints of non-vascularized grafts.

We propose RPNI and VDMT models in rabbits as potentially clinically relevant due to their practical advantages. Rabbits are manageable, easy to house, and relatively cost-effective compared to macaques. Furthermore, the size of rabbits facilitates the implementation of implantable devices for electrical activity recording and the investigation of the human-machine interface. The aim of this study was to develop and evaluate the first preclinical rabbit models of RPNI and VDMT as an exploratory methodological platform for translational research and future testing of implantable neuromuscular interfaces.

Material and methods

Animals

Three-month-old New Zealand rabbits (Granja San Bernardo, Madrid, Spain) were used (4 males, 4 females). Animals were randomly allocated to the RPNI (n = 4) or VDMT (n = 4) groups with balanced sex distribution (2 males and 2 females per group).

Rabbits were housed under standard temperature and light cycles with unrestricted access to food and water. All procedures were performed in accordance with European animal research legislation (Directive 2010/63/EU), minimizing pain and distress. The study was approved by the institutional ethics committee and the regional authority (PROEX 80.5/23, Madrid, Spain).

Experimental design

Eight rabbits were randomized as described above to receive either an RPNI (n = 4) or a VDMT (n = 4). The right hind limb received the assigned construct, while the contralateral limb served as an internal denervated control. Evaluations, including macroscopic inspection, nerve conduction studies, and histological analysis, were performed 8 weeks postoperatively. All animals were euthanized humanely with intravenous pentobarbital.

The sample size (n = 8) was intentionally minimal in accordance with European ethical restrictions and the "Three Rs" principles (Replacement, Reduction, Refinement). No formal sample size calculation was performed, as the study was designed as a pilot exploratory model in accordance with ethical principles of animal reduction. Accordingly, the study was designed to assess feasibility and generate preliminary data rather than to establish definitive comparative efficacy between constructs. Randomization and allocation concealment were performed by an investigator not involved in the surgical procedures. Histological and electrophysiological assessments were conducted by evaluators blinded to treatment allocation.

Anesthesia technique

All anesthetic procedures were performed by the same anesthesiologist (M.A.) to ensure protocol consistency. Rabbits were sedated intramuscularly with medetomidine (50 µg/kg) and ketamine (10 mg/kg). After 15 minutes, intravenous and arterial catheters were placed in the auricular vessels, followed by preoxygenation with 100% oxygen via face mask for 3–5 minutes. General anesthesia was induced with intravenous propofol (2–5 mg/kg) and maintained with continuous IV infusion (0.1–0.5 mL/kg/min). A rabbit-specific laryngeal mask was used with a Mapleson F system for oxygen delivery.

Analgesia included a lumbosacral epidural injection of bupivacaine (1 mg/kg) and morphine (0.1 mg/kg), using aseptic technique. Postoperative analgesia was maintained with meloxicam (1 mg/kg SC) and a fentanyl patch (25 µg/h) placed between the scapulae. Physiological monitoring included ECG, pulse oximetry, capnography, invasive arterial

pressure, and core temperature. Lactated Ringer's solution was administered at 5 mL/kg/h. Temperature was maintained between 37.5–38.5°C.

For electrophysiological assessments, the same sedation and monitoring protocol was applied. Atipamezole (100 µg/kg SC) was administered to facilitate recovery. Antibiotic prophylaxis (enrofloxacin) and analgesics were continued for five days post-procedure.

Surgical technique

All surgical procedures were performed by the same surgical team, composed of two surgeons, a neurophysiologist, and a veterinary specialist in anesthesia.

A. RPNI

In the right hind limb of each rabbit, a single RPNI was constructed using a free muscle graft harvested from the biceps femoris, measuring 15 × 5 × 2 mm (Fig. 1A). A longitudinal incision was made on the lateral distal thigh to expose the sciatic nerve within the biceps femoris. The peroneal nerve was carefully dissected and transected distally at its entry into the lateral and medial compartments below the stifle joint.

The muscle graft was harvested as demonstrated in Additional file 1 and transferred to the proximal stump of the peroneal nerve. The nerve stump was implanted into the muscle graft and secured using 6-0 Prolene® sutures (Ethicon, New Brunswick, NJ) (Fig. 1B and Additional File 2). The muscle was then wrapped around the nerve in a configuration analogous to the previously described "burrito" technique (Fig. 1C) [41].

For electrophysiological recording, one bipolar stainless steel needle electrode (Inomed, Emmendingen, Germany) was inserted intramuscularly within the RPNI (Fig. 1D). Electrodes were positioned approximately 2–3 mm into the center of the graft and secured to the epimysium with a 4-0 Vicryl stitch to prevent displacement. Electrode leads were tunneled and buried within the subcutaneous abdominal wall to ensure stability and minimize mechanical interference.

B. VDMT

In the right hind limb of each rabbit, a VDMT was constructed. A longitudinal incision was made along the lateral aspect of the leg, followed by meticulous dissection to expose the peroneal nerve and the gastrocnemius muscle [52]. The motor branch of the peroneal nerve was identified and confirmed by intraoperative electrical stimulation, then transected to achieve complete denervation. Successful muscle denervation was verified intraoperatively by the absence of contraction following proximal peroneal nerve stimulation.

A vascularized pedicled gastrocnemius muscle flap measuring 20 × 20 × 10 mm was subsequently elevated at the site corresponding to intraoperatively identified muscle contraction, with preservation of its native vascular supply. The distal stump of the transected motor branch was implanted into the thickness of the vascularized muscle flap (Fig. 2A) and secured using 6-0 Prolene® sutures (Ethicon, New Brunswick, NJ) (Fig. 2B; Additional File 3), in accordance with previously described VDMT techniques [47–51]. No recording electrode was implanted at the time of VDMT construction.

C. Control

In both groups, the contralateral (left) hind limb served as the control. An incision was made on the lateral thigh to expose the peroneal nerve. The peroneal nerve was carefully dissected, transected, and marked with a 6-0 Prolene® suture. The distal stump was left free and not coapted to prevent spontaneous reinnervation. A bipolar electrode (Inomed, Emmendingen, Germany) was implanted 2–3 mm into the center of the biceps femoris and secured to the epimysium with 4-0 Vicryl; electrode cables were buried subcutaneously in the abdominal wall.

No intraoperative complications were recorded. One rabbit developed postoperative paraparesis, likely related to epidural anesthesia, and was humanely euthanized. This animal was replaced to maintain the predefined sample size ($n = 8$).

Electrophysiology

Nerve conduction studies were performed 8 weeks postoperatively to evaluate reinnervation and signal detection in both RPNI and VDMT constructs. Lower temperature (35°C) was maintained during electrophysiological studies to ensure recording stability. All electrophysiological studies were performed by the same senior neurophysiologist (E.S.B.) to ensure methodological consistency.

In the RPNI group, the electrode leads were exposed through the abdominal wall. The peroneal nerve was carefully dissected. Motor nerve conduction (MNC) testing was performed by stimulating the proximal stump of the peroneal nerve with a concentric probe, always by the same neurophysiologist. Recorded parameters included presence of activity,

threshold, latency, amplitude, and the ability to isolate RPNI-specific signals. Equivalent assessments were performed in the contralateral denervated limb.

In the VDMT group, the construct was similarly exposed, and a bipolar intramuscular electrode was placed at the time of electrophysiological evaluation and connected to the recording system. MNC studies were performed using the same stimulation protocol and outcome measures. Control limbs were tested to confirm absence of reinnervation and to validate signal specificity.

Electrophysiological recordings were performed under standardized conditions using a subcutaneous needle electrode as ground to ensure signal stability and minimize electrical noise. Baseline activity was assessed prior to stimulation (amplified to 100 µV/division), confirming the absence of relevant electrical interference in all animals. Compound motor action potentials (CMAPs) were obtained by direct stimulation of the proximal stump of the peroneal nerve using a concentric probe, employing low-intensity thresholds (0.2–1.2 mA) to minimize activation of adjacent nerves and reduce crosstalk. Signals were considered valid when reproducible responses were obtained across repeated stimulations, with appropriate amplitude, latency, and waveform morphology consistent with muscle activation. Recordings lacking reproducibility, showing inconsistent morphology, or obtained in the presence of electrical noise were excluded from analysis.

Histology

For each rabbit, nerve–muscle units from RPNI and VDMT constructs, as well as denervated control muscles, were harvested for histopathological evaluation. Samples were fixed in 10%

buffered formalin for 24 hours, sectioned at 5 mm, embedded in paraffin, cut into 3 μm slices, and stained with hematoxylin–eosin.

Evaluated features included necrosis—quantified as a percentage (0–100%) of the total analyzed muscle area by microscopic examination using image-analysis software, with necrotic regions identified by established morphological criteria (loss of tissue architecture, hypereosinophilia, fragmentation/degeneration of muscle fibers, and associated inflammatory infiltrate) and delineated relative to apparently viable tissue, applying the same evaluation criteria across both experimental groups and with the evaluating pathologist blinded to group allocation—granulation tissue (absent or peripheral), inflammation (graded 0: absent; 1: mild—scattered foci; 2: moderate—multiple non-confluent foci; 3: severe—confluent foci), muscle fiber atrophy, and epimysial fibrosis (both classified as present or absent).

All histological analyses were performed by the same senior pathologist (A.G.P.) with extensive experience in peripheral nerve and muscle pathology. The parameters assessed—necrosis, inflammation, fibrosis, and muscle atrophy—are standard, well-validated criteria in RPNI and VDMT research and have demonstrated high reproducibility across previous studies [4,5,7,8,12,14,16–25].

Statistical analysis

Non-parametric tests were used to compare outcomes between groups: exact Mann–Whitney U test for quantitative necrosis (expressed as percentage of the analyzed muscle area) and inflammation, and Fisher’s exact test for fibrosis and neuroma formation. All tests were two-

sided, and significance was set at $p < 0.05$. Samples with complete necrosis were excluded from fibrosis analysis.

Statistical analyses were performed using SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA). Non-parametric methods were selected because of the small sample size and non-normal data distribution, as confirmed by the Shapiro–Wilk test [53].

Given the strict limitations on animal numbers in survival rabbit studies, no a priori power calculation was feasible; accordingly, this study was designed as an exploratory feasibility model.

Results

Macroscopic Evaluation

In the RPNI group (Fig. 3), the implanted muscle exhibited macroscopic signs of impaired viability, including pale coloration, poor vascularization, and visible atrophy (rabbits #2, #3, #4). Specimen #1 additionally demonstrated overt necrosis.

In contrast, VDMT constructs (Fig. 4) maintained a well-perfused appearance with preserved muscle volume and no gross evidence of necrosis or ischemic compromise.

Electrophysiology (Additional File 4)

In the RPNI group, no nerve-evoked electrical activity was recorded from any construct at 8 weeks. Although the control limb also showed no activity, as expected, proximal musculature generated reproducible signals, confirming appropriate system calibration and recording system integrity.

In the VDMT group, electrical activity was detected in three of four constructs. Rabbits #7 and #8 demonstrated clear and reproducible electrical responses (Fig. 5 and 6), while no activity was detected in rabbit #6. No electrical activity was observed in any VDMT control limb. A summary of electrophysiological outcomes is provided in Table 1.

Histological evaluation

In RPNI-treated limbs (Fig. 7 and 8), marked histopathological alterations were observed. Quantitative morphometric assessment showed a substantial necrotic muscle area in all RPNI specimens (rabbit #1: 95%; #2: 20%; #3: 30%; #4: 80%). Histologically, necrosis displayed a caseous necrotic pattern surrounded by intense inflammation and multinucleated giant cells, consistent with chronic tissue degeneration. Granulation tissue, when present, was located in the subepimysial region. All RPNI specimens exhibited inflammation (mild in #2; severe in #1 and #4), peripheral muscle atrophy, and epimysial fibrosis (rabbits #2, #3, and #4). Traumatic neuromas were identified in all RPNI cases.

In VDMT-treated limbs (Fig. 9 and 10), histological changes were considerably less severe. No necrosis was detected in any specimen (0% necrotic area in all cases). Granulation tissue appeared in the subepimysial region of rabbits #5, #7, and #8. Inflammation was mild in rabbit #5 and absent in #6, #7, and #8, suggesting a reduced immune response. Peripheral muscle atrophy was observed in all VDMT constructs but was less pronounced than in RPNIs, indicating better preservation of muscle architecture. Epimysial fibrosis was present only in rabbit #5. Traumatic neuromas were visualized in rabbits #5 and #6, occurring less frequently and appearing less extensive than in the RPNI group.

Control limbs exhibited mild inflammatory changes and muscle atrophy, with no evidence of

necrosis or neuroma formation. A summary of histological findings for both groups is provided in Table 2.

Statistical analysis

Between-group comparisons showed a significantly higher percentage of muscle necrosis in the RPNI group compared with the VDMT group (RPNI: median [IQR], 55% [25–87.5] vs VDMT: 0% [0–0]; exact Mann–Whitney U test, U = 16, p = 0.0286). Inflammation remained higher in the RPNI group, showing a nonsignificant trend (p = 0.14). Epimysial fibrosis was also more frequent in RPNIs but did not reach statistical significance (p = 0.14). No significant difference in neuroma formation was observed between groups (p = 0.4).

Discussion

To our knowledge, this exploratory study represents the first attempt to establish a rabbit model for both RPNI and VDMT. A major translational challenge for these constructs is the need for implantable devices capable of reliably recording electromyographic (EMG) signals. The anatomical scale of the rabbit accommodates components that approximate human implant dimensions, enabling integration and testing of near–clinical grade interfaces. Ultimately, the goal is to develop a compact implantable system, similar in size to a pacemaker [54], capable of stable signal acquisition and transmission. In this context, the rabbit provides a scalable and suitable platform for evaluating the performance and biocompatibility of bioelectronic devices. Therefore, this work should be interpreted primarily as a feasibility and methodological study designed to establish a scalable rabbit platform, rather than as a definitive comparative efficacy study between both constructs.

Taken together, these findings position the rabbit as a valuable intermediate model between small-animal studies and clinical translation.

This study further supports the relevance of rabbits in biomedical research due to their anatomical and inflammatory similarities to humans. Their phylogenetic proximity to primates and larger muscle mass offer advantages over rodent models for human–machine interface studies [55,56]. RPNI constructs have been extensively evaluated in rats [4,5,8,10,12,14,16–41] and macaques [25,42,43] and our recent systematic review [7] showed that approximately 80% of published work originated from the University of Michigan.

In the present study, RPNIs in rabbits showed limited tissue vascularization and poor viability. Several factors may explain this outcome. First, the biceps femoris, selected for its accessibility, has relatively high metabolic and oxygen demands [57,58], which may hinder early graft revascularization in a non-vascularized construct. Second, placing the bipolar electrode immediately after suturing the RPNI may have partially interfered with early revascularization and tissue integration, promoting localized fibrosis and compromising the regenerative microenvironment required for graft survival [59]. We avoided electrode insertion at 8 weeks (and prefer the insertion at the initial RPNI-surgery) to prevent compromising the graft's vascular integrity, as isolating the muscle after 8 weeks for the placement of the electrode might disrupt neovascularization and yield false-negative electrophysiological results. Finally, the Michigan group proposed an alternative inlay technique in rats, showing improved outcomes compared to the traditional 'burrito' method [41]. Although the burrito technique has been successful in rats [4,5,8,10,12,14,16–26,29–32,37,39–41], macaques [42,43] and humans [6,9,48,51,60–101]; it may not be optimal for rabbit anatomy.

Given the limited viability and insufficient vascularization of RPNIs in this model, we implemented a vascularized alternative (VDMT). This strategy ensures continuous perfusion even in the absence of innervation, improving graft survival and overcoming the size constraints of free muscle grafts. Although VDMTs have been described in other species [7,47–51], this is the first application in rabbits.

Several technical differences between RPNI and VDMT constructs may have influenced the observed outcomes and limit direct comparability between both models. These include the immediate implantation of electrodes in RPNIs but not in VDMTs, the use of a non-vascularized muscle graft versus a vascularized pedicled flap, and the relatively large size and high metabolic demand of the biceps femoris [57,58]. In RPNI constructs, electrodes were implanted at the time of construction to avoid potential devascularization or disruption of the graft during the secondary dissection required at the 8-week evaluation. These factors should therefore be considered when interpreting the comparative results.

Muscle size and metabolic demand may play a critical role in construct viability, particularly in non-vascularized models. RPNI constructs rely on diffusion and secondary neovascularization during the early postoperative phase, and therefore may be more susceptible to failure when using larger muscles with high metabolic demand. Experimental evidence has shown that increasing muscle graft mass in RPNI models is associated with reduced graft viability and increased necrosis [10], highlighting the limitations of non-vascularized muscle constructs. In our model, the muscle graft was harvested as thin as technically feasible. In contrast, VDMT constructs are based on vascularized pedicled flaps, providing immediate perfusion and potentially reducing dependence on muscle size or

metabolic requirements. These differences likely reflect inherent biological distinctions between both constructs rather than muscle selection alone.

The Burrito-RPNI technique was selected in this study as it represents one of the most widely used and historically established approaches in preclinical models. As reported in previous systematic analyses [7], this technique has been consistently employed across a substantial number of experimental studies. Although different RPNI configurations have been described in the literature [41], the aim of the present study was not to compare surgical techniques but rather to establish a reproducible and scalable rabbit model. Future studies should directly evaluate the impact of different RPNI configurations on functional and histological outcomes.

Electrophysiology

The VDMT constructs displayed stable and consistent electrical activity with adequate amplitude at the 8-week endpoint, with the exception of rabbit #6. Because no necrosis or inflammatory changes were identified on histological analysis, this isolated absence of signal is most likely attributable to a technical factor (such as suboptimal electrode positioning or insufficient nerve–muscle contact) or to early neuroma formation. Rabbit #8 also demonstrated increased latency, suggesting delayed or incomplete maturation of the reinnervating axons. Together, these findings indicate that VDMT reinnervation may show some variability under the present experimental conditions [7,47–51].

Importantly, although neuromuscular junction maturation may not be complete at the 8-week timepoint, the detection of reproducible electrophysiological signals in the majority of VDMT constructs suggests that at least early-stage functional reinnervation had already

occurred. These findings should therefore be interpreted as reflecting an early stage of neuromuscular integration rather than full maturation, while supporting the feasibility of detecting construct-specific signals within this timeframe. Conversely, the absence of detectable EMG activity in RPNI constructs may also be partly attributable to the relatively short follow-up period rather than true construct failure, as longer time intervals may be required to achieve complete neuromuscular maturation and stable signal generation.

Several explanations may account for this variability. First, muscle reinnervation may not have occurred, or a longer postoperative interval may be required for functional maturation. Second, the limited muscle volume inherent to the model makes it technically challenging to obtain a clean, isolated signal. Electrical noise and crosstalk from adjacent muscles may obscure the true signal, increasing the risk of false-positive or misleading recordings.

Accurate interpretation therefore requires strict confirmation that recorded potentials originate from the transected nerve and not from surrounding tissues. This includes verifying appropriate amplitude, duration, and latency in response to stimulation, as well as reproducibility across repeated trials, which is particularly important in distinguishing true construct-derived signals from background muscle activity.

Rabbit #6, which did not show detectable electrophysiological activity, was male; however, given the limited sample size, no conclusions can be drawn regarding the influence of sex on the observed variability.

Histology (Fig. 11 and 12)

The histopathological findings revealed clear differences in tissue response between RPNI and VDMT constructs. Although both groups exhibited inflammatory and fibrotic changes, the severity and distribution of these features diverged considerably, highlighting potential limitations of RPNI relative to VDMT under the present experimental conditions.

Importantly, quantitative morphometric assessment demonstrated significantly greater muscle necrosis in RPNIs compared with VDMTs ($p = 0.0286$). This finding is particularly relevant, as graft necrosis represents a critical limiting factor for long-term signal stability and implantable interface performance in large-animal models.
Neither model demonstrated flawless histological outcomes; however, VDMT specimens consistently displayed better tissue preservation, with no detectable necrosis across all samples.
In contrast, RPNIs exhibited substantial necrosis, accompanied by inflammation and epimysial fibrosis, supporting the notion that non-vascularized free grafts struggle to survive in a large-animal setting. The biceps femoris was selected as the donor muscle due to its accessibility and predictable anatomy. However, its relatively large volume and high metabolic demand [57,58] may pose challenges for early graft integration, particularly in RPNIs, which rely initially on diffusion-based oxygenation prior to neovascularization. Although alternative donor muscles were not assessed in this study, such intrinsic characteristics may have contributed to the poor viability observed in several RPNI constructs. Future studies should evaluate muscle grafts with lower metabolic requirements to enhance free-graft survival in large-animal models.

Two VDMT constructs showed traumatic neuroma formation (rabbits #5 and #6), whereas the remaining two did not. Because neuroma development is a common consequence of nerve

transection, it is plausible that all VDMTs developed some degree of neuromatous tissue, but that the affected region was not captured within the histological plane of sectioning in all cases. Only rabbit #6 did not show consistent electrical activity. It is possible that the neuroma observed in rabbit #5 allowed some axonal ingrowth into the muscle, which may have been sufficient to generate detectable electrical activity.

Macroscopic identification of neuroma formation was limited by the need to preserve construct integrity for histological analysis, as exposure of the distal nerve end would have required dismantling the constructs. Therefore, neuroma assessment was primarily based on histological evaluation, which provides a more sensitive and specific assessment of neural architecture.

Limitations

Although rabbits share relevant anatomical and physiological features with humans, several limitations must be acknowledged when interpreting the translational value of these findings.

Firstly, the small sample size (n = 8), imposed by strict ethical regulations mandating reduction of animal use in survival studies, inherently limits statistical power and may mask biologically meaningful differences between groups.

Furthermore, some aspects of neuromuscular junction maturation may require longer observation periods; prior studies report significant maturation around 10–12 weeks [8,9], whereas the present study assessed constructs at 8 weeks. Consequently, the present work should be regarded as a preliminary feasibility investigation that provides foundational insight into early RPNI and VDMT behavior in rabbits. Larger studies with extended follow-up will be essential to confirm these findings and refine the translational relevance of both

constructs. For these reasons, the present findings should be interpreted within the context of an exploratory and methodological framework.

Operative time and intraoperative blood loss were not systematically recorded, as they were not predefined outcome measures in this exploratory study. However, all procedures were completed within approximately one hour (RPNI ~45 minutes; VDMT ~35 minutes), and intraoperative blood loss was minimal and did not require specific management. These parameters should be formally evaluated in future studies.

These limitations should be considered when interpreting the results and their potential clinical implications.

Future Directions and Translational Perspective

This study highlights the feasibility of detecting construct-specific electrical signals in a rabbit model, establishing a solid foundation for future translational work.

The anatomical scale of the rabbit accommodates implantable devices with dimensions and configurations comparable to those intended for human use, enabling a more clinically relevant evaluation of emerging bioelectronic interface technologies than is possible in small-animal models. The encouraging biological behavior observed in VDMTs supports continued investigation into long-term reinnervation, functional activation, prosthetic integration, and improved signal specificity under near-clinical conditions. Future studies should incorporate delayed electrode placement, optimized muscle selection, and extended follow-up to refine construct performance, assess long-term signal stability, and better replicate clinical implantation scenarios.

From a clinical perspective, both RPNI and VDMT constructs may play a relevant role in the management of peripheral nerve injuries, particularly in the context of limb amputation. By providing a physiological target for regenerating axons, these interfaces may contribute to the prevention and control of symptomatic neuromas after amputation. At the same time, their ability to generate stable and detectable electrophysiological signals supports their potential use in the development of advanced myoelectric prosthetic control strategies, where reliable biological signal sources are required to improve functional outcomes.

Conclusions

This investigation presents the first exploratory rabbit model allowing preclinical evaluation of RPNI and VDMT constructs in a large-animal setting. VDMTs demonstrated superior structural preservation, including the absence of detectable necrosis with quantitative morphometric assessment, and more consistent electrophysiological activity at 8 weeks. However, these findings should be interpreted cautiously given the pilot design, small sample size, and technical variables. Larger studies incorporating expanded cohorts and longer follow-up intervals will be essential to determine the comparative performance and translational potential of both constructs. The present findings should be interpreted within the exploratory and methodological nature of the study.

List of abbreviations

RPNI: Regenerative Peripheral Nerve Interface, VDMT: Vascularized Denervated Muscle Target, EMG: Electromyography, HMI: Human–machine interface, ISCIII: Instituto de Salud Carlos III.

Declarations

Ethics approval and consent to participate

All experimental procedures were conducted in accordance with European legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU). The study protocol was approved by the institutional animal ethics committee and the regional authority of the Community of Madrid (PROEX 80.5/23, Madrid, Spain). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by Instituto de Salud Carlos III (ISCIII) through the project PI22/00862 and co-funded by the European Union. The funding bodies had no role in the study design, data collection and analysis, interpretation of data, or writing of the manuscript.

Authors' contributions

JGP, AAM and LC conceived and designed the study. JGP and AAM performed the surgical procedures. MA acted as the veterinary specialist and anesthesiologist, being responsible for animal welfare, anesthesia protocols, and perioperative management. ESB conducted the electrophysiological analyses. AGP performed the histological evaluation. MCM performed the statistical analysis. RG, JDM, and EA contributed to the technical and engineering aspects of the study. All authors contributed to data interpretation, manuscript drafting, and approved the final version of the manuscript.

Acknowledgements

The authors thank the veterinary and technical staff involved in animal care and perioperative management for their support during the study.

References

1. Ajiboye AB, Willett FR, Young DR, Memberg WD, Murphy BA, Miller JP, et al. Restoration of reaching and grasping movements through brain-controlled muscle stimulation in a person with tetraplegia: a proof-of-concept demonstration. Lancet [Internet]. 2017;389:1821–30. Available from: http://dx.doi.org/10.1016/S0140-6736(17)30601-3

2. Shahriari D, Rosenfeld D, Anikeeva P. Emerging Frontier of Peripheral Nerve and Organ Interfaces. Neuron [Internet]. 2020;108:270–85. Available from: http://dx.doi.org/10.1016/j.neuron.2020.09.025

3. Aman M, Bergmeister KD, Festin C, Sporer ME, Russold MF, Gstoettner C, et al. Experimental Testing of Bionic Peripheral Nerve and Muscle Interfaces: Animal Model Considerations. Front Neurosci [Internet]. 2019;13:1442. Available from: http://dx.doi.org/10.3389/fnins.2019.01442

4. Kung TA, Langhals NB, Martin DC, Johnson PJ, Cederna PS, Urbanchek MG. Regenerative peripheral nerve interface viability and signal transduction with an implanted electrode. Plast Reconstr Surg [Internet]. 2014;133:1380–94. Available from: http://dx.doi.org/10.1097/PRS.0000000000000168

5. Sando IC, Leach MK, Woo SL, Moon JD, Cederna PS, Langhals NB, et al. Regenerative Peripheral Nerve Interface for Prostheses Control: Electrode Comparison. J Reconstr Microsurg [Internet]. 2016;32:194–9. Available from: http://dx.doi.org/10.1055/s-0035-1565248

6. Kubiak CA, Kemp SWP, Cederna PS. Regenerative peripheral nerve interface for management of postamputation neuroma. JAMA Surg [Internet]. 2018;153:681–2. Available from: http://dx.doi.org/10.1001/jamasurg.2018.0864

7. González-Prieto J, Cristóbal L, Arenillas M, Giannetti R, Muñoz Frías JD, Alonso Rivas E, et al. Regenerative Peripheral Nerve Interfaces (RPNIs) in Animal Models and Their Applications: A Systematic Review. Int J Mol Sci [Internet]. 2024;25. Available from: http://dx.doi.org/10.3390/ijms25021141

8. Frost CM, Ursu DC, Flattery SM, Nedic A, Hassett CA, Moon JD, et al. Regenerative peripheral nerve interfaces for real-time, proportional control of a Neuroprosthetic hand. J Neuroeng Rehabil [Internet]. 2018;15:108. Available from: http://dx.doi.org/10.1186/s12984-018-0452-1

9. Vu PP, Vaskov AK, Irwin ZT, Henning PT, Lueders DR, Laidlaw AT, et al. A regenerative peripheral nerve interface allows real-time control of an artificial hand in upper limb amputees. Sci Transl Med. 2020;12.

10. Hu Y, Ursu DC, Sohasky RA, Sando IC, Ambani SLW, French ZP, et al. Regenerative peripheral nerve interface free muscle graft mass and function. Muscle Nerve [Internet]. 2021;63:421–9. Available from: http://dx.doi.org/10.1002/mus.27138

11. Baldwin J, Moon JD, Cederna PS, Urbanchek MG. Abstract 99. Plast Reconstr Surg [Internet]. 2012;130:73. Available from: http://journals.lww.com/00006534-201207001-00101

12. Svientek SR, Ursu DC, Cederna PS, Kemp SWP. Fabrication of the Composite Regenerative Peripheral Nerve Interface (C-RPNI) in the Adult Rat. J Vis Exp [Internet]. 2020; Available from: http://dx.doi.org/10.3791/60841

13. Urbanchek MG, Wei B, Baghmanli Z, Sugg K, Cederna PS. Long-Term Stability of Regenerative Peripheral Nerve Interfaces (RPNI). Plast Reconstr Surg [Internet]. 2011 [cited 2024 Feb 6];128:88. Available from: http://journals.lww.com/00006534-201110001-00114

14. Woo SL, Urbanchek MG, Leach MK, Moon JD, Cederna P, Langhals NB. Quantification of muscle-derived signal interference during monopolar needle electromyography of a peripheral nerve interface in the rat hind limb. Conf Proc IEEE Eng Med Biol Soc [Internet]. 2014;2014:4382–5. Available from: http://dx.doi.org/10.1109/EMBC.2014.6944595

15. Urbanchek MG, Moon JD, Sugg KB, Langhals NB, Cederna PS, Baghmanli Z. Abstract 2P. Plast Reconstr Surg [Internet]. 2012;130:84. Available from: http://journals.lww.com/00006534-201207001-00118

16. Ursu DC, Urbanchek MG, Nedic A, Cederna PS, Gillespie RB. In vivo characterization of regenerative peripheral nerve interface function. J Neural Eng [Internet]. 2016;13:026012. Available from: http://dx.doi.org/10.1088/1741-2560/13/2/026012

17. Kubiak CA, Svientek SR, Dehdashtian A, Lawera NG, Nadarajan V, Bratley JV, et al. Physiologic signaling and viability of the muscle cuff regenerative peripheral nerve interface (MC-RPNI) for intact peripheral nerves. J Neural Eng [Internet]. 2021;18. Available from: http://dx.doi.org/10.1088/1741-2552/ac1b6b

18. Ursu D, Nedic A, Urbanchek M, Cederna P, Gillespie RB. Adjacent regenerative peripheral nerve interfaces produce phase-antagonist signals during voluntary walking in rats. J Neuroeng Rehabil [Internet]. 2017;14:33. Available from: http://dx.doi.org/10.1186/s12984-017-0243-0

19. Frost CM, Cederna PS, Martin DC, Shim BS, Urbanchek MG. Decellular biological scaffold polymerized with PEDOT for improving peripheral nerve interface charge transfer. Conf Proc IEEE Eng Med Biol Soc [Internet]. 2014;2014:422–5. Available from: http://dx.doi.org/10.1109/EMBC.2014.6943618

20. Langhals NB, Woo SL, Moon JD, Larson JV, Leach MK, Cederna PS, et al. Electrically

stimulated signals from a long-term Regenerative Peripheral Nerve Interface. Conf Proc IEEE Eng Med Biol Soc [Internet]. 2014;2014:1989–92. Available from: http://dx.doi.org/10.1109/EMBC.2014.6944004

21. Svientek SR, Wisely JP, Dehdashtian A, Bratley JV, Cederna PS, Kemp SWP. The Muscle Cuff Regenerative Peripheral Nerve Interface for the Amplification of Intact Peripheral Nerve Signals. J Vis Exp [Internet]. 2022; Available from: http://dx.doi.org/10.3791/63222

22. Frost CM, Wei B, Baghmanli Z, Cederna PS, Urbanchek MG. PEDOT electrochemical polymerization improves electrode fidelity and sensitivity. Plast Reconstr Surg [Internet]. 2012;129:933–42. Available from: http://dx.doi.org/10.1097/PRS.0b013e31824422bf

23. French ZP, Carrothers NS, Hassett CA, Moon JD, Langhals NB, Cederna PS, et al. Abstract 61: Characterization of regenerative peripheral nerve device signaling during evoked maximal and submaximal fatiguing conditions. Plast Reconstr Surg [Internet]. 2014;133:72. Available from: http://dx.doi.org/10.1097/01.prs.0000445094.07094.de

24. Woo S, Urbanchek MG, Leach MK, Moon JD, Cederna PS, Langhals NB. Utilizing nonvascularized partial skeletal muscle grafts in peripheral nerve interfaces for prosthetic control. J Am Coll Surg [Internet]. 2014 [cited 2023 Aug 23];219:e136. Available from: https://journals.lww.com/journalacs/citation/2014/10001/utilizing_nonvascularized_partial_skeletal_muscle.326.aspx

25. Sando IC, French ZP, Hassett CA, Moon JD, Langhals NB, Cederna PS, et al. Regenerative Peripheral Nerve Signal During Fatigue Conditions. J Am Coll Surg [Internet]. 2014 [cited 2023 Aug 23];219:S88. Available from: https://journals.lww.com/journalacs/citation/2014/09001/regenerative_peripheral_nerve_sign

al_during.182.aspx

26. Sando IC, Adidharma W, Nedic A, Ursu DC, Mays EA, Hu Y, et al. Dermal Sensory Regenerative Peripheral Nerve Interface for Reestablishing Sensory Nerve Feedback in Peripheral Afferents in the Rat. Plast Reconstr Surg [Internet]. 2023;151:804e–13e. Available from: http://dx.doi.org/10.1097/PRS.0000000000010086

27. Wang Z, Zhang D, Yi XZ, Zhao Y, Yu A. Effects of regenerative peripheral nerve interface on dorsal root ganglia neurons following peripheral axotomy. Front Neurosci [Internet]. 2022;16. Available from: https://www.frontiersin.org/articles/10.3389/fnins.2022.914344

28. Wu J, Zhang Y, Zhang X, Lin Z, Li G. Regenerative Peripheral Nerve Interfaces Effectively Prevent Neuroma Formation After Sciatic Nerve Transection in Rats. Front Mol Neurosci [Internet]. 2022;15:938930. Available from: http://dx.doi.org/10.3389/fnmol.2022.938930

29. Urbanchek MG, Kung TA, Frost CM, Martin DC, Larkin LM, Wollstein A, et al. Development of a Regenerative Peripheral Nerve Interface for Control of a Neuroprosthetic Limb. Biomed Res Int [Internet]. 2016;2016:5726730. Available from: http://dx.doi.org/10.1155/2016/5726730

30. Nedic A, Ursu D, Moon JD, Hassett CA, Gillespie RB, Langhals NB, et al. Abstract 60: Signal strength, reliability, and validity of active regenerative peripheral nerve interface device operation during voluntary movement. Plast Reconstr Surg [Internet]. 2014;133:71. Available from: http://dx.doi.org/10.1097/01.prs.0000445093.99469.d4

31. Frost CM, Ursu D, Nedic A, Hassett CA, Moon JD, Gillespie B, et al. Abstract 18: real-time proportional control of a neuroprosthetic hand by a rodent regenerative peripheral nerve

interface. Plast Reconstr Surg [Internet]. 2014;133:27–8. Available from: http://dx.doi.org/10.1097/01.prs.0000445051.00569.56

32. Larson JV, Urbanchek MG, Moon JD, Hunter DA, Newton P, Johnson PJ, et al. Abstract 17: prototype sensory regenerative peripheral nerve interface for artificial limb somatosensory feedback. Plast Reconstr Surg [Internet]. 2014;133:26–7. Available from: http://dx.doi.org/10.1097/01.prs.0000445040.57505.da

33. Spearman BS, Kuliasha CA, Judy JW, Schmidt CE. Integration of flexible polyimide arrays into soft extracellular matrix-based hydrogel materials for a tissue-engineered electronic nerve interface (TEENI). J Neurosci Methods [Internet]. 2020;341:108762. Available from: http://dx.doi.org/10.1016/j.jneumeth.2020.108762

34. Wang Z, Yi X-Z, Yu A-X. Regenerative peripheral nerve interface prevents neuroma formation after peripheral nerve transection. Neural Regeneration Res [Internet]. 2023;18:814–8. Available from: http://dx.doi.org/10.4103/1673-5374.353498

35. Atkinson EW, Kuliasha CA, Kasper M, Furniturewalla A, Lim AS, Jiracek-Sapieha L, et al. Examining thein vivofunctionality of the magnetically aligned regenerative tissue-engineered electronic nerve interface (MARTEENI). J Neural Eng [Internet]. 2022;19. Available from: http://dx.doi.org/10.1088/1741-2552/ac8bfe

36. Lacour SP, Fitzgerald JJ, Lago N, Tarte E, McMahon S, Fawcett J. Long micro-channel electrode arrays: a novel type of regenerative peripheral nerve interface. IEEE Trans Neural Syst Rehabil Eng [Internet]. 2009;17:454–60. Available from: http://dx.doi.org/10.1109/TNSRE.2009.2031241

37. Baghmanli Z, Urbanchek MG, Wei B, Sugg KB, Kuzonv WM, Cederna PS. Neurotization of freely transferred muscle grafts in a regenerative peripheral nerve interface.

J Am Coll Surg [Internet]. 2011 [cited 2023 Aug 23];213:S98. Available from: https://journals.lww.com/journalacs/citation/2011/09001/neurotization_of_freely_transferred_muscle_grafts.218.aspx

38. Kim B., Reyes A., Garza B., Ibarra E., Luna R., Flores D., Choi Y. A microchannel neural interface with microwires for recording and stimulating peripheral nerves [Internet]. 2014. Available from: http://dx.doi.org/10.1111/ner.12232

39. Senger J-LB, Hardy P, Thorkelsson A, Duia S, Hsiao R, Kemp SWP, et al. A Direct Comparison of Targeted Muscle Reinnervation and Regenerative Peripheral Nerve Interfaces to Prevent Neuroma Pain. Neurosurgery [Internet]. 2023; Available from: http://dx.doi.org/10.1227/neu.0000000000002541

40. Dehdashtian A, Timek JH, Svientek SR, Risch MJ, Bratley JV, Riegger AE, et al. Sexually Dimorphic Pattern of Pain Mitigation Following Prophylactic Regenerative Peripheral Nerve Interface (RPNI) in a Rat Neuroma Model. Neurosurgery [Internet]. 2023; Available from: http://dx.doi.org/10.1227/neu.0000000000002548

41. Senger J-L, Thorkelsson A, Wang BY, Chan KM, Kemp SWP, Webber CA. “Inlay” Regenerative Peripheral Nerve Interface (RPNI) is superior to “Burrito” RPNI for successful treatment of rat neuroma pain. Plast Reconstr Surg [Internet]. 2023; Available from: http://dx.doi.org/10.1097/PRS.0000000000010911

42. Irwin ZT, Schroeder KE, Vu PP, Tat DM, Bullard AJ, Woo SL, et al. Chronic recording of hand prosthesis control signals via a regenerative peripheral nerve interface in a rhesus macaque. J Neural Eng [Internet]. 2016;13:046007. Available from: http://dx.doi.org/10.1088/1741-2560/13/4/046007

43. Vu PP, Irwin ZT, Bullard AJ, Ambani SW, Sando IC, Urbanchek MG, et al. Closed-loop

continuous hand control via chronic recording of regenerative peripheral nerve interfaces. IEEE Trans Neural Syst Rehabil Eng [Internet]. 2018;26:515–26. Available from: http://dx.doi.org/10.1109/tnsre.2017.2772961

44. Foote RH, Carney EW. The rabbit as a model for reproductive and developmental toxicity studies. Reprod Toxicol [Internet]. 2000;14:477–93. Available from: http://dx.doi.org/10.1016/s0890-6238(00)00101-5

45. Cai B, Wang N. Large Animal Stroke Models vs. Rodent Stroke Models, Pros and Cons, and Combination? Acta Neurochir Suppl [Internet]. 2016;121:77–81. Available from: http://dx.doi.org/10.1007/978-3-319-18497-5_13

46. Milani-Nejad N, Janssen PML. Small and large animal models in cardiac contraction research: advantages and disadvantages. Pharmacol Ther [Internet]. 2014;141:235–49. Available from: http://dx.doi.org/10.1016/j.pharmthera.2013.10.007

47. Khoo KHL, Suresh V, Lee E, Harris T, Glass C, Tuffaha S. Vascularized Denervated Muscle Targets: A Comparison with Regenerative Peripheral Nerve Interfaces to Determine Association Between Muscle Graft Size and Pain. Plastic and Reconstructive Surgery--Global Open [Internet]. 2022;10:16–7. Available from: https://journals.lww.com/prsgo/fulltext/2022/10001/vascularized_denervated_muscle_targets__a.24.aspx

48. Suresh V, Schaefer EJ, Calotta NA, Giladi AM, Tuffaha SH. Use of Vascularized, Denervated Muscle Targets for Prevention and Treatment of Upper-Extremity Neuromas. J Hand Surg Glob Online [Internet]. 2023;5:92–6. Available from: http://dx.doi.org/10.1016/j.jhsg.2022.06.001

49. Tuffaha SH, Glass C, Rosson G, Shores J, Belzberg A, Wong A. Vascularized,

Denervated Muscle Targets: A Novel Approach to Treat and Prevent Symptomatic Neuromas. Plast Reconstr Surg Glob Open [Internet]. 2020;8:e2779. Available from: http://dx.doi.org/10.1097/GOX.0000000000002779

50. Calotta NA, Hanwright PJ, Giladi A, Tuffaha SH. Vascularized, Denervated Muscle Targets for Treatment of Symptomatic Neuromas in the Upper Extremity: Description of Operative Technique. Tech Hand Up Extrem Surg [Internet]. 2022;26:141–5. Available from: http://dx.doi.org/10.1097/BTH.0000000000000374

51. Valerio I, Schulz SA, West J, Westenberg RF, Eberlin KR. Targeted Muscle Reinnervation Combined with a Vascularized Pedicled Regenerative Peripheral Nerve Interface. Plast Reconstr Surg Glob Open [Internet]. 2020;8:e2689. Available from: http://dx.doi.org/10.1097/GOX.0000000000002689

52. Mukhopadhyay S, Wagner LR. Rabbit Anatomy: A Brief Photographic Atlas and Dissection Guide - Part I: Muscular System (Third Edition). 2023 [cited 2025 Jan 18]; Available from: https://open.clemson.edu/rabbit/2

53. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). Biometrika [Internet]. 1965;52:591–611. Available from: https://doi.org/10.1093/biomet/52.3-4.591

54. Gstoettner C, Festin C, Prahm C, Bergmeister KD, Salminger S, Sturma A, et al. Feasibility of a Wireless Implantable Multi-electrode System for High-bandwidth Prosthetic Interfacing: Animal and Cadaver Study. Clin Orthop Relat Res [Internet]. 2022;480:1191–204. Available from: http://dx.doi.org/10.1097/CORR.0000000000002135

55. Graur D, Duret L, Gouy M. Phylogenetic position of the order Lagomorpha (rabbits, hares and allies). Nature [Internet]. 1996;379:333–5. Available from:

http://dx.doi.org/10.1038/379333a0

56. Fischer B, Chavatte-Palmer P, Viebahn C, Navarrete Santos A, Duranthon V. Rabbit as a reproductive model for human health. Reproduction [Internet]. 2012;144:1–10. Available from: http://dx.doi.org/10.1530/REP-12-0091

57. Korthuis RJ. Skeletal Muscle Circulation. Colloq Ser Integr Syst Physiol Mol Funct [Internet]. 2011 [cited 2025 Jan 18];3:1–144. Available from: https://pubmed.ncbi.nlm.nih.gov/21850766/

58. Chen M-M, Li Y, Deng S-L, Zhao Y, Lian Z-X, Yu K. Mitochondrial function and reactive oxygen/nitrogen species in skeletal muscle. Front Cell Dev Biol [Internet]. 2022 [cited 2025 Jan 18];10:826981. Available from: https://pubmed.ncbi.nlm.nih.gov/35265618/

59. Sohal HS, Clowry GJ, Jackson A, O'Neill A, Baker SN. Mechanical flexibility reduces the foreign body response to long-term implanted microelectrodes in rabbit cortex. PLoS One [Internet]. 2016;11:e0165606. Available from: http://dx.doi.org/10.1371/journal.pone.0165606

60. Gfrerer L, Wong FK, Hickle K, Eberlin KR, Valerio IL, Austen WG Jr. RPNI, TMR, and reset neurectomy/relocation nerve grafting after nerve transection in headache surgery. Plast Reconstr Surg Glob Open [Internet]. 2022;10:e4201. Available from: http://dx.doi.org/10.1097/GOX.0000000000004201

61. Kubiak CA, Adidharma W, Kung TA, Kemp SWP, Cederna PS, Vemuri C. Decreasing postamputation pain with the Regenerative Peripheral Nerve Interface (RPNI). Ann Vasc Surg [Internet]. 2022;79:421–6. Available from: http://dx.doi.org/10.1016/j.avsg.2021.08.014

62. Pejkova S, Nikolovska B, Srbov B, Tusheva S, Jovanoski T, Jovanovska K, et al.

Prophylactic regenerative peripheral nerve interfaces in elective lower limb amputations. Pril (Makedon Akad Nauk Umet Odd Med Nauki) [Internet]. 2022;43:41–8. Available from: http://dx.doi.org/10.2478/prilozi-2022-0004

63. Morag Y, Ganesh Kumar N, Hamill JB, Cederna PS, Masotti M, Kemp SWP, et al. Ultrasound appearance of regenerative peripheral nerve interface with clinical correlation. Skeletal Radiol [Internet]. 2023;52:1137–57. Available from: http://dx.doi.org/10.1007/s00256-022-04256-6

64. Sayegh A, Jaloux C, Witters M, Mayoly A, Kachouh N. Update on upper limb neuroma management. J Craniofac Surg [Internet]. 2023;34:1140–3. Available from: http://dx.doi.org/10.1097/SCS.0000000000009164

65. Lee C, Vaskov AK, Gonzalez MA, Vu PP, Davis AJ, Cederna PS, et al. Use of regenerative peripheral nerve interfaces and intramuscular electrodes to improve prosthetic grasp selection: a case study. J Neural Eng [Internet]. 2022;19. Available from: http://dx.doi.org/10.1088/1741-2552/ac9e1c

66. Gonzalez MA, Vu PP, Vaskov AK, Cederna PS, Chestek CA, Gates DH. Characterizing sensory thresholds and intensity sensitivity of Regenerative Peripheral Nerve Interfaces: A Case Study. IEEE Int Conf Rehabil Robot [Internet]. 2022;2022:1–6. Available from: http://dx.doi.org/10.1109/ICORR55369.2022.9896481

67. Shamoun F, Shamoun V, Akhavan A, Tuffaha SH. Target receptors of regenerating nerves: Neuroma formation and current treatment options. Front Mol Neurosci [Internet]. 2022;15:859221. Available from: http://dx.doi.org/10.3389/fnmol.2022.859221

68. Richards JT, Baird MD, Tintle SM, Souza JM, Renninger CH, Potter BK. Peripheral nerve management in extremity amputations. Orthop Clin North Am [Internet]. 2022;53:155–

66. Available from: http://dx.doi.org/10.1016/j.ocl.2022.01.002

69. de Lange JWD, Hundepool CA, Power DM, Rajaratnam V, Duraku LS, Zuidam JM. Prevention is better than cure: Surgical methods for neuropathic pain prevention following amputation - A systematic review. J Plast Reconstr Aesthet Surg [Internet]. 2022;75:948–59. Available from: http://dx.doi.org/10.1016/j.bjps.2021.11.076

70. Ganesh Kumar N, Kung TA, Cederna PS. Regenerative peripheral nerve interfaces for advanced control of upper extremity prosthetic devices. Hand Clin [Internet]. 2021;37:425–33. Available from: http://dx.doi.org/10.1016/j.hcl.2021.04.005

71. Ganesh Kumar N, Kung TA. Regenerative peripheral nerve interfaces for the treatment and prevention of neuromas and neuroma pain. Hand Clin [Internet]. 2021;37:361–71. Available from: http://dx.doi.org/10.1016/j.hcl.2021.05.003

72. Bhashyam AR, Liu Y, Kao DS. Targeted peripheral nerve interface: Case report with literature review. Plast Reconstr Surg Glob Open [Internet]. 2021;9:e3532. Available from: http://dx.doi.org/10.1097/GOX.0000000000003532

73. Hoyt BW, Gibson JA, Potter BK, Souza JM. Practice patterns and pain outcomes for targeted muscle reinnervation: An informed approach to targeted muscle reinnervation use in the acute amputation setting. J Bone Joint Surg Am [Internet]. 2021;103:681–7. Available from: http://dx.doi.org/10.2106/JBJS.20.01005

74. Gstoettner C, Laengle G, Salminger S, Festin C, Platzgummer H, Aszmann OC. Der chirurgische Umgang mit peripheren Nerven nach Extremitätenverlust. Orthopade [Internet]. 2021;50:14–23. Available from: http://dx.doi.org/10.1007/s00132-020-04032-1

75. Hobusch GM, Döring K, Brånemark R, Windhager R. Advanced techniques in

amputation surgery and prosthetic technology in the lower extremity. EFORT Open Rev [Internet]. 2020;5:724–41. Available from: http://dx.doi.org/10.1302/2058-5241.5.190070

76. Kurlander DE, Wee C, Chepla KJ, Lineberry KD, Long TC, Gillis JA, et al. TMRpni: Combining two peripheral nerve management techniques. Plast Reconstr Surg Glob Open [Internet]. 2020;8:e3132. Available from: http://dx.doi.org/10.1097/GOX.0000000000003132

77. Lans J, Hoftiezer Y, Lozano-Calderón SA, Heng M, Valerio IL, Eberlin KR. Risk factors for neuropathic pain following major upper extremity amputation. J Reconstr Microsurg [Internet]. 2021;37:413–20. Available from: http://dx.doi.org/10.1055/s-0040-1718547

78. Hooper RC, Cederna PS, Brown DL, Haase SC, Waljee JF, Egeland BM, et al. Regenerative peripheral nerve interfaces for the management of symptomatic hand and digital neuromas. Plast Reconstr Surg Glob Open. 2020;8.

79. Santosa KB, Oliver JD, Cederna PS, Kung TA. Regenerative peripheral nerve interfaces for prevention and management of neuromas. Clin Plast Surg [Internet]. 2020;47:311–21. Available from: http://dx.doi.org/10.1016/j.cps.2020.01.004

80. Woo SL, Kung TA, Brown DL, Leonard JA, Kelly BM, Cederna PS. Regenerative peripheral nerve interfaces for the treatment of postamputation neuroma pain: A pilot study. Plast Reconstr Surg Glob Open [Internet]. 2016;4:e1038. Available from: http://dx.doi.org/10.1097/gox.0000000000001038

81. Chou J, Liston JM, DeGeorge BR. Traditional neuroma management strategies: A systematic review. Ann Plast Surg [Internet]. 2023;90:S350–5. Available from: http://dx.doi.org/10.1097/SAP.0000000000003342

82. Yang A, Thompson RW. Pilot feasibility study of a simple regenerative peripheral nerve

interface designed to diminish cutaneous dysesthesia after supraclavicular operations. J Vasc Surg Cases Innov Tech [Internet]. 2022;8:287–92. Available from: http://dx.doi.org/10.1016/j.jvscit.2022.03.013

83. Vu PP, Lu CW, Vaskov AK, Gates DH, Gillespie RB, Kemp SWP, et al. Restoration of proprioceptive and cutaneous sensation using regenerative peripheral nerve interfaces in humans with upper limb amputations. Plast Reconstr Surg [Internet]. 2022;149:1149e–54e. Available from: http://dx.doi.org/10.1097/PRS.0000000000009153

84. Bruce WJ, Brown AL, Romanelli MR, Mailey BA. Autologous muscle-derived nerve wrap for prevention of symptomatic microneuromas in primary nerve repair. Cureus [Internet]. 2022;14:e22513. Available from: http://dx.doi.org/10.7759/cureus.22513

85. Chang BL, Mondshine J, Fleury CM, Attinger CE, Kleiber GM. Incidence and nerve distribution of symptomatic neuromas and phantom limb pain after below-knee amputation. Plast Reconstr Surg [Internet]. 2022;149:976–85. Available from: http://dx.doi.org/10.1097/PRS.0000000000008953

86. Wade SM, Harrington CJ, Hoyt BW, Melendez-Munoz AM, Potter BK, Souza JM. Beyond limb salvage: Limb restoration efforts following remote combat-related extremity injuries optimize outcomes and support sustained surgical readiness. Mil Med [Internet]. 2023;188:e584–90. Available from: http://dx.doi.org/10.1093/milmed/usab403

87. Starr BW, Chung KC. Traditional neuroma management. Hand Clin [Internet]. 2021;37:335–44. Available from: http://dx.doi.org/10.1016/j.hcl.2021.04.002

88. Schwentker AR. Cautious enthusiasm: The role of targeted muscle reinnervation and regenerative peripheral nerve interface in the treatment of nerve-related amputation pain: Commentary on an article by Benjamin W. hoyt, MD, et al.: “practice patterns and pain

outcomes for targeted muscle reinnervation. An informed approach to targeted muscle reinnervation use in the acute amputation setting." J Bone Joint Surg Am [Internet]. 2021;103:e34. Available from: http://dx.doi.org/10.2106/JBJS.21.00096

89. Geary M, Gaston RG, Loeffler B. Surgical and technological advances in the management of upper limb amputees. Bone Joint J [Internet]. 2021;103-B:430–9. Available from: http://dx.doi.org/10.1302/0301-620X.103B3.BJJ-2020-1184.R1

90. Souza JM, Wade SM, Harrington CJ, Potter BK. Functional limb restoration through amputation: Minimizing pain and optimizing function with the use of advanced amputation techniques. Ann Surg [Internet]. 2021;273:e108–13. Available from: http://dx.doi.org/10.1097/SLA.0000000000003942

91. Dellon AL, Aszmann OC. In musculus, veritas? Nerve "in muscle" versus targeted muscle reinnervation versus regenerative peripheral nerve interface: Historical review. Microsurgery [Internet]. 2020;40:516–22. Available from: http://dx.doi.org/10.1002/micr.30575

92. Kubiak CA, Kemp SWP, Cederna PS, Kung TA. Prophylactic regenerative peripheral nerve interfaces to prevent postamputation pain. Plast Reconstr Surg [Internet]. 2019;144:421e–30e. Available from: http://dx.doi.org/10.1097/PRS.0000000000005922

93. Eberlin KR, Ducic I. Surgical algorithm for neuroma management: A changing treatment paradigm. Plast Reconstr Surg Glob Open [Internet]. 2018;6:e1952. Available from: http://dx.doi.org/10.1097/GOX.0000000000001952

94. Nghiem BT, Sando IC, Gillespie RB, McLaughlin BL, Gerling GJ, Langhals NB, et al. Providing a sense of touch to prosthetic hands. Plast Reconstr Surg [Internet]. 2015;135:1652–63. Available from: http://dx.doi.org/10.1097/PRS.0000000000001289

95. Chang BL, Kleiber GM. Below-the-knee amputation with targeted muscle reinnervation: Operative technique and technical pearls. Plast Reconstr Surg Glob Open [Internet]. 2023;11:e4663. Available from: http://dx.doi.org/10.1097/GOX.0000000000004663

96. Mauch JT, Kao DS, Friedly JL, Liu Y. Targeted muscle reinnervation and regenerative peripheral nerve interfaces for pain prophylaxis and treatment: A systematic review. PM R [Internet]. 2023;15:1457–65. Available from: http://dx.doi.org/10.1002/pmrj.12972

97. Vu PP, Vaskov AK, Lee C, Jillala RR, Wallace DM, Davis AJ, et al. Long-term upper-extremity prosthetic control using regenerative peripheral nerve interfaces and implanted EMG electrodes. J Neural Eng [Internet]. 2023;20. Available from: http://dx.doi.org/10.1088/1741-2552/accb0c

98. Pettersen E, Sassu P, Reinholdt C, Dahm P, Rolfson O, Björkman A, et al. Surgical treatments for postamputation pain: study protocol for an international, double-blind, randomised controlled trial. Trials [Internet]. 2023;24:304. Available from: http://dx.doi.org/10.1186/s13063-023-07286-0

99. Xu W, Toyoda Y, Lin IC. Upper extremity prosthetics: Current options and future innovations. J Hand Surg Am [Internet]. 2023;48:1034–44. Available from: http://dx.doi.org/10.1016/j.jhsa.2023.05.018

100. Leach GA, Dean RA, Kumar NG, Tsai C, Chiarappa FE, Cederna PS, et al. Regenerative peripheral nerve interface surgery: Anatomic and technical guide. Plast Reconstr Surg Glob Open [Internet]. 2023;11:e5127. Available from: http://dx.doi.org/10.1097/GOX.0000000000005127

101. Lin Z, Yu P, Chen Z, Li G. Regenerative peripheral nerve interface reduces the incidence of neuroma in the lower limbs after amputation: a retrospective study based on

ultrasound. J Orthop Surg Res [Internet]. 2023;18:619. Available from: http://dx.doi.org/10.1186/s13018-023-04116-6

Table legend

Table 1. Electrophysiological results of RPNI and VDMT at 8 Weeks.

Table 2. Histopathological analysis of RPNI and VDMT at 8 Weeks.

Figure legend

Figure 1. (A) Donor muscle graft harvested from the right biceps femoris muscle for the RPNI construction. Example of RPNI. (B) The proximal stump of the peroneal nerve is sutured into the free muscle graft; an arrow indicates the site of nerve-to-muscle coaptation. (C) The free muscle graft is sutured in a manner described as a "burrito". (D) Location of the bipolar recording electrode in the RPNI.

Figure 2. Example of VDMT. (A) The distal end of the motor branch was placed within the thickness of the vascularized muscle flap; the triangle indicates the vascularized muscle flap, and the arrow identifies the motor branch used to construct the VDMT. (B) The vascularized muscle flap is sutured with the nerve inside.

Figure 3. Macroscopic findings of the RPNI (#1 to #4) at 8 weeks. The asterisk (*) indicates the RPNI muscle graft, and the star (★) identifies the nerve used for its construction.

Figure 4. Macroscopic findings of the VDMT (#5 to #8) at 8 weeks. The asterisk (*) indicates the VDMT muscle graft, and the star (★) identifies the nerve used for its construction.

Figure 5. Example of electrophysiology analysis from VDMT in rabbit #7. A response is present with CMAP (Compound Muscle Action Potential) recording after nerve stimulation. The threshold is low (1 mA). The amplitude after supramaximal stimulation is 0.63 mV.

Figure 6. Example of electrophysiology analysis from VDMT in rabbit #8. A response is present with CMAP recording after nerve stimulation. The threshold is very low (0.6 mA). The amplitude after supramaximal stimulation is 2.162 mV. Note increased latency in all traces (≥4 ms), suggesting demyelination.

Figure 7. Histological assessment of muscle injury in rabbits subjected to regenerative peripheral nerve interface (RPNI) (#1–#4). Histological sections are organized by animal (rows) and pathological features (columns), including necrosis, inflammation, neuroma formation, and normal control muscle. Non-evaluable samples are indicated when extensive tissue necrosis precluded reliable assessment of specific histological parameters.

Figure 8. Histological assessment of tissue remodeling in rabbits subjected to regenerative peripheral nerve interface (RPNI) (#1–#4). Histological sections are organized by animal (rows) and pathological features (columns), including granulation tissue, fiber atrophy, epimysial fibrosis, and normal control muscle. Non-evaluable samples are indicated when extensive tissue necrosis precluded reliable assessment of specific histological parameters.

Figure 9. Histological assessment of muscle injury in rabbits subjected to vascularized denervated muscle targets (VDMT) (#5–#8). Histological sections are organized by animal (rows) and pathological features (columns), including necrosis, inflammation, neuroma formation, and normal control muscle.

Figure 10. Histological assessment of tissue remodeling in rabbits subjected to vascularized denervated muscle targets (VDMT) (#5–#8). Histological sections are organized by animal (rows) and pathological features (columns), including granulation tissue, fiber atrophy, epimysial fibrosis, and normal control muscle.

Figure 11. Histological comparison: Mean histologic scores for RPNI and VDMT constructs at 8 weeks. Granulation tissue (0–1), inflammation (0–3), fiber atrophy (0–1), epimysial fibrosis (0–1), and neuroma (0–1) were evaluated using semiquantitative ordinal scales. For each parameter, the bar height represents the mean score across available specimens in each group (RPNI vs VDMT). Error bars indicate the standard deviation (SD), reflecting inter-animal variability in histologic involvement. n = 4 per group.

Figure 12. Quantitative assessment of muscle necrosis in RPNI and VDMT constructs at 8 weeks. Each data point represents an individual animal. RPNIs exhibited a significantly greater necrotic area compared with VDMTs (exact two-sided Mann–Whitney U test, $p = 0.0286$).

Additional files

Additional file 1

- File format: MP4
- Title: RPNI construction: harvesting of the muscle graft

- Description: Video demonstrating the harvesting of a free muscle graft from the right biceps femoris muscle for Regenerative Peripheral Nerve Interface (RPNI) construction.

Additional file 2

- File format: MP4
- Title: RPNI construction: nerve implantation into the muscle graft
- Description: Video showing the implantation of the proximal stump of the peroneal nerve into the free muscle graft during RPNI construction.

Additional file 3

- File format: MP4
- Title: VDMT construction: nerve coaptation to a vascularized muscle flap
- Description: Video illustrating Vascularized Denervated Muscle Target (VDMT) construction, in which the proximal end of the motor branch of the peroneal nerve is sutured into a vascularized muscle flap derived from the previously denervated gastrocnemius muscle.

Additional file 4

- File format: MP4
- Title: Electrophysiological assessment of VDMT in a rabbit model
- Description: Video showing an example of a neurophysiological study in rabbit #7. Compound muscle action potentials (CMAPs) recorded after low-intensity nerve stimulation demonstrate a stable and highly reproducible response, despite low signal amplitude.