

PROCEEDINGS OF SPIE

SPIDigitalLibrary.org/conference-proceedings-of-spie

The bionic man: Not too far away!

Stephen W. P. Kemp, Melanie G. Urbanchek, Zachary T. Irwin, Cynthia A. Chestek, Paul S. Cederna

Stephen W. P. Kemp, Melanie G. Urbanchek, Zachary T. Irwin, Cynthia A. Chestek, Paul S. Cederna, "The bionic man: Not too far away!," Proc. SPIE 10194, Micro- and Nanotechnology Sensors, Systems, and Applications IX, 1019415 (18 May 2017); doi: 10.1117/12.2264130

SPIE.

Event: SPIE Defense + Security, 2017, Anaheim, California, United States

Development of Regenerative Peripheral Nerve Interfaces for Motor Control of Neuroprosthetic Devices

Stephen W.P. Kemp^{a,b}, Melanie G. Urbanek^a, Zachary T. Irwin^b, Cynthia A. Chestek^b,
Paul S. Cederna^{a,b}

^a Department of Surgery, Section of Plastic and Reconstructive Surgery, University of Michigan, 1500 E Medical Center Drive, Ann Arbor, MI, 48109-5895; ^b

Department of Surgery, Section of Plastic and Reconstructive Surgery, University of Michigan, Ann Arbor, MI.

ABSTRACT

Traumatic peripheral nerve injuries suffered during amputation commonly results in debilitating neuropathic pain in the affected limb. Modern prosthetic technologies allow for intuitive, simultaneous control of multiple degrees of freedom. However, these state-of-the-art devices require separate, independent control signals for each degree of freedom, which is currently not possible. As a result, amputees reject up to 75% of myoelectric devices preferring instead to use body-powered artificial limbs which offer subtle sensory feedback. Without meaningful and intuitive sensory feedback, even the most advanced myoelectric prostheses remain insensate, burdensome, and are associated with enormous cognitive demand and mental fatigue. The ideal prosthetic device is one which is capable of providing intuitive somatosensory feedback essential for interaction with the environment. Critical to the design of such a bioprosthetic device is the development of a reliable biologic interface between human and machine. This ideal patient-prosthetic interface allows for transmission of both afferent somatosensory information and efferent motor signals for a closed-loop feedback system of neural control. Our lab has developed the Regenerative Peripheral Nerve Interface (RPNI) as a biologic nerve interface designed for stable integration of a prosthetic device with transected peripheral nerves in a residual limb. The RPNI is constructed by surgically implanting the distal end of a transected peripheral nerve into an autogenous muscle graft. Animal experiments in our lab have shown recording of motor signals from RPNI's implanted into both rodents and monkeys. Here, we achieve high amplitude EMG signals with a high signal to noise (SNR) ratio.

Keywords: nerve injury, regeneration, regenerative peripheral nerve interface, prosthetic control, neuroma

INTRODUCTION

Nerve injury is a significant disease burden in the industrialized countries worldwide with the economic impact of treating such injuries in the US alone estimated to be \$18 billion^{1, 2}. Despite the capacity of peripheral nerves to regenerate their axons following injury, functional outcomes in patients are frequently poor^{3, 4}. Nerve injury is significant in people who have undergone amputation of a limb. One in 190 Americans has an amputated limb, with over 185,000 new amputations performed annually⁵. Currently, the prevalence of major limb loss is predicted to be 1.6 million, and this number is projected to increase to over 3.6 million by 2050⁶. While there have been impressive advancements in the field of neuroprosthetics for restoring extremity function after amputation, the ideal prosthesis has yet to be realized. The medical and engineering communities have championed the development of complex prosthetic limbs capable of multiple joint manipulations⁷ with the potential to emulate intricate functions of the native extremity⁸. However, these advanced devices are not intuitive to use since the patient must rely entirely on visual cues instead of sensory feedback to control the prosthetic⁹.

Unlike forearm amputations, where myoelectric signals from remaining muscles can be isolated to power a prosthetic hand, high amputations of the upper arm remain a difficult functional challenge to the patient. Currently, targeted muscle reinnervation has been introduced for select upper extremity amputees, but this method is dependent on surface electromyogram (EMG) signals to link the reinnervated muscle and the prosthetic limb¹⁰. However, this interface exhibits signal instability,

interference, and requires daily pattern recognition calibration by a computer^{11, 12}. Furthermore, although this technique is currently the best form of prosthetic control available, it is difficult with this approach to achieve high fidelity motor function or high fidelity sensory feedback. Therefore, in an effort to optimize signal strength and fidelity, recent work has concentrated on a variety of peripheral nerve interfaces that have been developed to bridge the gap between bioelectric and mechanical signals¹³. These include non-invasive devices such as extraneural cuff electrodes^{14, 15}, and invasive options such as flexible nerve plates¹⁶, intrafascicular electrodes^{13, 17}, and penetrating electrodes^{18, 19}. However, these constructs are limited by several major shortcomings. For example, scarring and nerve encapsulation remain a substantial problem with conductive materials introduced into a peripheral nerve²⁰. Furthermore, these interfaces do not account for the inherent ability of the transected peripheral nerve to continue sprouting and form neuromas, which can be painful for the patient and creates signal interference for the neuroprosthesis.

The Regenerative Peripheral Nerve Interface (RPNI) is a novel strategy for motor control of neuroprosthetic devices in transected peripheral nerves in an animal model of major limb amputation. The RPNI consists of a residual peripheral nerve that is implanted into a free skeletal muscle graft after excision of a terminal neuroma bulb (Figure 1). Because the muscle fibers within the free graft are both nonvascularized and denervated, they undergo a process of degeneration followed by regeneration and are subsequently reinnervated by the sprouting axons of the implanted nerve. In contrast to existing methods of surgical intervention, the RPNI encourages the formation of new neuromuscular junctions within the free muscle graft, thereby greatly reducing the number of axons at the end of the nerve without a functional connection and reducing the chance of neuroma recurrence. Multiple RPNIs can be performed within the residual limb using a piece of free muscle graft for each available peripheral nerve end. The following study examined the use of RPNIs for prosthetic control in both rodents and non-human primate (macaque) animal models.

Regenerative Peripheral Nerve Interface

Step 1

- A divided peripheral nerve end is identified after amputation injury.
- If a painful neuroma exists, the sensitive terminal end is excised.

Step 2

- A free muscle graft is harvested from the subject.
- The end of the nerve is implanted into the substance of the muscle.

Step 3

- The muscle is wrapped around the nerve and sutured closed.
- Axons will regenerate within the RPNI and reinnervate the muscle.

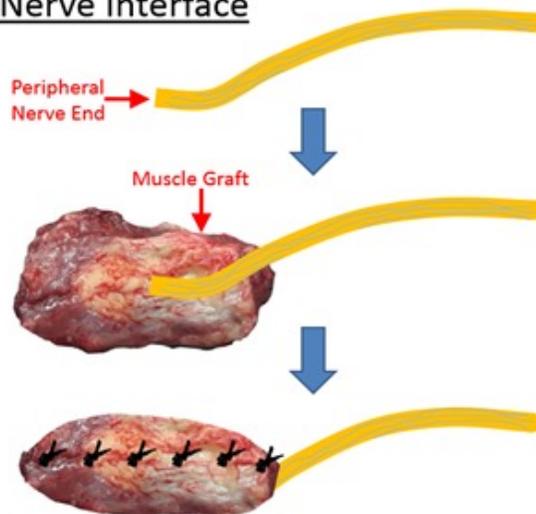


Figure 1. Schematic of a surgical RPNI construct for motor control of a neuroprosthetic.

MATERIALS AND METHODS

Animals

All procedures were approved by the University of Michigan University Committee on the use and care of animals. Fisher 344 rats were used for our rodent studies, while Rhesus macaques were used for our non-human primate studies. All surgical interventions were carried out using inhalational anesthetic (2% Isoflurane in 98% oxygen; Halocarbon Laboratories, River Edge, NJ). All animals were administered Rimadyl (0.3 mL/100 g body weight (Pfizer) for post-operative pain relief. All animals were maintained in a temperature and humidity controlled environment and all animals were allowed food and water *ad libitum*, with a 12:12 h light:dark cycle. All surgical procedures were carried out in an aseptic manner under an

operating microscope (Leitz, Willowdale, ON). All animals were sacrificed at study termination under deep anaesthesia with Euthanol (sodium pentobarbital, 240 mg/mL concentration, 1 mL/kg, Bimeda-MTC, Cambridge, ON) administered intracardially.

Surgical Procedure

[i] Fisher 344 Rats

Each regenerative peripheral nerve interface was fabricated in the same fashion while the rat was deeply anesthetized using intraperitoneal pentobarbital sodium. An incision was made on the lateral aspect of the left hind limb, and intramuscular dissection proceeded through the biceps femoris muscle until the common peroneal nerve was encountered. The peroneal nerve was meticulously dissected and then sharply divided at its entrance into the lateral compartment of the lower leg. The left extensor digitorum longus muscle (EDL) was exposed and its attachments were incised to elevate it out of the anterior compartment using handheld cautery to divide the pedicle. The muscle was then freely transferred into the ipsilateral thigh in its original orientation and secured by means of its tendons to the femur and fibular head using 7-0 Prolene (Johnson & Johnson Co., San Lorenzo, Puerto Rico) sutures. A superficial myotomy was created through the epimysium of the EDL at the junction of its proximal and middle thirds to accommodate neurotisation by the peroneal nerve. Several interrupted 9-0 nylon sutures secured the epineurium of the peroneal nerve to the epimysium of the transferred EDL. The surgical site was closed in layers.

[ii] Rhesus Macaques

First, the distal end of the target peripheral nerve is identified, isolated, and, if necessary, dissected into smaller branches or individual fascicles. For each resulting nerve, a small muscle graft, approximately 1×3 cm, is harvested from any healthy, native donor muscle. The distal end of each nerve is then placed centrally in its corresponding muscle graft and secured in place with sutures from epineurium to epimysium. The muscle graft is then folded around the nerve to create a stable housing and sutured together. Implantation of multiple RPNIs is achieved by making small access incisions over the nerves of interest and the muscle for harvesting grafts. The above procedure is then simply repeated as necessary to create the desired number of RPNIs. Once implanted, RPNIs can be placed anywhere in the limb, but in most cases would be left at the original site of the nerve ending in order to minimize anatomical disruption. Following these procedures, we implanted a total of nine RPNIs on separate branches of the median and radial nerves in the forearms of two rhesus macaques L and R. These branches terminated on the extrinsic finger flexors and extensors, providing a basis for prosthetic hand control. To preserve motor function, we transected only minor, redundant terminal motor nerve branches.

Electrophysiology

For our rodent studies, testing began 1 month after regenerative peripheral nerve interface surgery, and continued on a monthly basis. Rats were anesthetized with isoflurane and recording occurred through an electrophysiologic monitoring system (Natus Medical, Inc., San Carlos, Calif.). A stainless steel ground electrode (Chalgren Enterprises, Gilroy, Calif.) was placed into the third web space of the foot, and another was inserted subcutaneously over the left hip as the reference. Insertional needle electromyography was performed to follow reinnervation of the EDL muscle after neurotization. This was accomplished by first identifying the position of the regenerative peripheral nerve interface by palpation and then briskly inserting a 26-gauge concentric electromyography needle (Natus Medical) percutaneously into the regenerative peripheral nerve interface muscle. The contralateral EDL was tested in a similar fashion. Findings indicative of persistent denervation of the regenerative peripheral nerve interface muscle included increased insertional activity (amplitude and duration compared with the control), fibrillation potentials, and positive sharp waves. Regenerative peripheral nerve interface muscles (regardless of electrode type) and control muscles demonstrating any of these indicators were counted each month and the frequency of these signs of denervation for each group was expressed as percentages.

Nerve conduction studies were performed on the regenerative peripheral nerve interface by means of bipolar percutaneous needle stimulation of the peroneal nerve with recording of the resultant compound muscle action potential in the regenerative peripheral nerve interface. The peroneal nerve was stimulated in two locations: distally near the regenerative peripheral nerve interface and proximally at the sciatic notch. The stimulus (1 pulse per second, 0.1-msec duration) was incrementally increased manually to

identify the stimulation required to generate a maximal compound muscle action potential. Similarly, nerve conduction studies were carried out on the control muscle with a recording needle electrode inserted into the contralateral EDL.

During the first RPNI implantation surgery in rhesus macaques, we implanted several bipolar epimysial EMG electrodes (Plastics One). The electrodes consisted of insulated stainless steel leads attached to a silicone backing. The electrodes were placed on the surface of the RPNI muscle grafts and secured in place by wrapping small intestinal submucosa (SIS) around the muscle-electrode construct and suturing it together. The leads were then tunneled subcutaneously along the arm and back to a connector on the animal's headcap. Leads were looped at the RPNIs and at each joint for strain relief. Shortly after surgery, the animal was able to break the leads at the margin of the headcap, leaving no intact electrodes for recording. In a revision surgery, it was noted that the stiffness of the silicone patch had caused significant scar formation and presumably impeded RPNI regeneration, so the epimysial electrodes were extracted and not used further on either monkey.

Prior to chronic electrode implantation in both animals (during epimysial electrode extraction in the first animal and during the initial implantation surgery in the second animal), RPNIs were placed superficially in the subcutaneous plane in order to facilitate acute, percutaneous recording. During task behavior, we recorded EMG from the superficial RPNIs via fine-wire electrodes (Natus Medical). The RPNIs were located using surface landmarks and surgical photos. The wires were inserted into the RPNI muscle via hypodermic needle. As the RPNIs were located directly subcutaneously, the needle was inserted at a shallow angle and advanced just far enough to bury both contacts under the skin in order to avoid contact with the muscle within the deep compartments. Recording locations were verified in further revision surgeries. Percutaneous recordings of healthy, intact muscles were also obtained for comparison.

To subsequently facilitate chronic recording of RPNI activity, we implanted bipolar intramuscular electrodes (IMMES, Ardiem Medical). The IM-MES electrodes consist of two insulated stainless steel leads coiled in a double helix formation and potted in silicone tubing. Contacts are formed by exposing the leads and wrapping them around the tubing, and a polypropylene anchor at the distal end secures the electrode in the muscle. In the first animal, the two contacts on the electrode were 4 mm long with a diameter of 1.27 mm (the diameter of the silicone tubing), and were separated by 6 mm. After noting that, in some cases, this was too large to fit both contacts within the muscle belly of an RPNI, a reduced contact size of 1.5 mm and inter-contact spacing of 2.5 mm were used for the second animal. A single IM-MES electrode was placed in the muscle belly of each RPNI, as well as in a healthy control muscle, by making a small incision and manually feeding the electrode anchor-first into the muscle. Leads were tunneled subcutaneously to a transcutaneous port on the animal's back and attached to a connector protected by a primate jacket.

During task performance, EMG signals from the RPNIs were input into either a DAM50 differential EMG amplifier (WPI), which filtered the signal between 10 and 1000 Hz with a gain of 1000x, or directly into a Cerebus neural signal processor (Blackrock Microsystems), which filtered the signal between 3 and 7000 Hz (unity gain). For real-time signal analysis, the Cerebus was used to record from multiple electrodes simultaneously. The DAM50 was used for lower noise recordings of a single electrode. In both cases, the processed signal was digitized and saved to disk by the Cerebus at 30 ks⁻¹. The signal was further sent from the Cerebus to the behavioral rig via ethernet, where it could be processed in real-time.

During several revision surgeries after RPNI maturation in both animals, we tested the mature RPNIs for reinnervation and tissue health by evoking compound muscle action potentials (CMAPs) via stimulation of the implanted nerve. Using a Teca Synergy evoked potential system (Viasys Healthcare), we either stimulated the nerve just proximal to the point of entry to the RPNI or stimulated the muscle of the RPNI itself while simultaneously recording from bipolar electrodes in the belly of the RPNI muscle. Stimulation parameters varied between surgeries, consisting primarily of a pulse width of 200 μ s and current amplitude between 1 and 20 mA when stimulating the nerve directly and a pulse width of 20 or 200 μ s and current amplitude between 30 and 60 mA when stimulating the nerve through the RPNI muscle.

Immunohistochemistry

Following electrophysiological assessment, the regenerative peripheral nerve interface muscle, and peroneal nerve were removed. The contralateral control EDL was also harvested. Tissues were preserved for histology and transmission electron microscopic imaging. Immunohistochemical analysis for neurofilament 200 and [alpha]-bungarotoxin enabled identification of axons and motor end plates, respectively. Activated macrophage response was visualized by immunostaining with ED1.

RESULTS

RPNI muscles survived long-term implantation in a rat model, and showed preservation of whole muscle structure after regeneration and subsequent reinnervation (Fig.1A). The muscle serves to both amplify neural signals and provides a viable site for axons to sprout and restore mature neuromuscular junctions. Histologic analysis of the RPNIs revealed several important findings: (1) muscle fibers comprising the free muscle grafts robustly regenerate within several weeks after implantation; (2) the implanted peripheral nerve ends from new neuromuscular junctions within the free muscle grafts (Fig.2B), and; (3) no evidence of neuroma was detected within the RPNIs.

Our success in rodent models led to RPNI experiments in a non-human primate model. Multiple RPNIs were implanted into two rhesus macaque monkeys and extensive electrophysiological tests were successfully conducted for up to 20 months (Fig.1C). These electrophysiological studies established that the neuromuscular junctions within the RPNIs are functional and that action potentials can be reliably transduced through the RPNI from the nerve into the muscle graft. This study further demonstrated that: (1) RPNI implantation did not result in any adverse effects in the two macaque subjects; (2) all RPNI free muscle grafts displayed robust regeneration and reinnervation; (3) RPNIs successfully transduced volitional high fidelity motor signals from divided motor nerve fascicles, indicating that functional neuromuscular junctions were formed within the RPNIs (Fig.1D), and; (4) no evidence of neuromas were found on histologic examination.

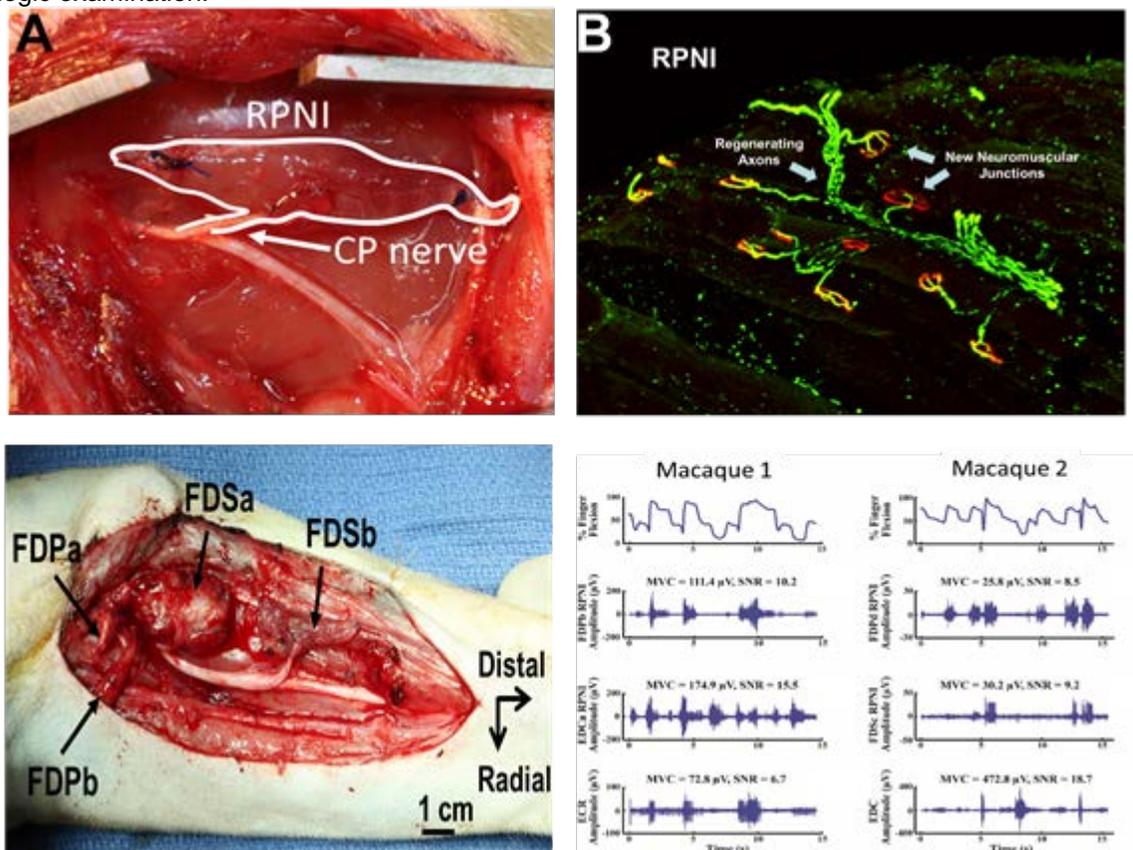


Figure 2. RPNI for motor control of neuroprosthetic devices. **A.** In situ rat RPNI: Extensor digitorum longus muscle (EDL) transferred to ipsilateral thigh and reinnervated with common peroneal nerve (RPNI outlined in white). **B.** Rat RPNI stained with alpha-bungarotoxin displaying

histological nerve regeneration and neuromuscular junction (NMJ) formation (axons in green, motor endplates in yellow, and new NMJs in red). **C.** In situ monkey RPNI (FDP = flexor digitorum profundus; FDS = flexor digitorum superficialis). **D.** EMG signals from rhesus macaque RPNIs display alternating patterns of EMG activation corresponding to hand flexion and extension.

CONCLUSIONS

The studies presented here show that RPNI grafts are successfully revascularized, regenerate, and become reinnervated after transfer to the end of a divided nerve. It takes approximately 3 months for an RPNI to mature and at that time, the muscle grafts have a mass similar to that of healthy control muscles. We have observed reliable EMG from these muscle constructs for up to at least 20 months post-implantation, which means that RPNI constructs still function in aged rats. Our results with two macaque monkeys further demonstrated the reliability and feasibility of RPNI constructs in animal models. These signals can be used for voluntary prosthetic control in a freely moving animal. These EMG signals recorded from RPNIs have high amplitude and a high signal-to-noise ratio.

We believe that our preclinical animal data presented here supports translation of the RPNI technique into human subjects for the purpose of utilizing RPNIs for prosthetic control in patients who have sustained major limb amputations. Our future work involves implanting multiple RPNIs into 5 patients with limb loss. Using ultrasound guidance, we will be able to place percutaneous fine-wire electrodes into each RPNIs to record volitional motor signals from each individual RPNI. We believe that we are capable of achieving very large efferent motor signals for very fine dexterous movements. As in our animal models, we hope to observe individual RPNIs amplify signals by 10-15 fold providing high signal-to-noise (SNR) ratio recording.

The results presented here are extremely encouraging, however, the next critical step is the development of a single interface that is capable of transferring afferent sensory information *and* efferent motor signals for intuitive prosthetic control. This interface would be especially ideal for patients with residual mixed sensorimotor nerve fascicles. In this way, we expect mixed peripheral nerves to regenerate in a target specific manner with sensory axons reinnervating dermal tissues and motor axons reinnervating muscle. This innate axonal regenerative specificity permits spatially distinct signal transduction and stimulation within the RPNI. Furthermore, this preferential distribution would be particularly useful for patients with proximal amputations where mixed sensorimotor nerve fascicles are more common.

References

- 1 Wojtkiewicz DM, Saunders J, Domeshek L, Novak CB, Kaskutas V, Mackinnon SE. Social impact of peripheral nerve injuries. *Hand (N Y)* 2015; 10: 161-7
- 2 Dias JJ, Garcia-Elias M. Hand injury costs. *Injury* 2006; 37: 1071-7
- 3 Lundborg G. *Nerve Injury and Repair*. New York: Churchill Livingstone, 2004
- 4 Akel BS, Oksuz C, Oskay D, Firat T, Tarakci E, Leblebicioglu G. Health-related quality of life in children with obstetrical brachial plexus palsy. *Qual. Life Res* 2013
- 5 Ziegler-Graham K, MacKenzie EJ, Ephraim PL, Travison TG, Brookmeyer R. Estimating the prevalence of limb loss in the United States: 2005 to 2050. *Arch Phys Med Rehabil* 2008; 89: 422-9
- 6 Owings M, Kozak, L.J. *Ambulatory and inpatient procedures in the United States*. U.S. Dept of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics 1998
- 7 Hijjawi JB, Kuiken TA, Lipschutz RD, Miller LA, Stubblefield KA, Dumanian GA. Improved myoelectric prosthesis control accomplished using multiple nerve transfers. *Plast Reconstr Surg* 2006; 118: 1573-8
- 8 Pylatiuk C, Schulz S, Doderlein L. Results of an Internet survey of myoelectric prosthetic hand users. *Prosthet Orthot Int* 2007; 31: 362-70

- 9 Biddiss EA, Chau TT. Upper limb prosthesis use and abandonment: a survey of the last 25 years. *Prosthet Orthot Int* 2007; 31: 236-57
- 10 Kuiken TA, Li G, Lock BA, Lipschutz RD, Miller LA, Stubblefield KA, Englehart KB. Targeted muscle reinnervation for real-time myoelectric control of multifunction artificial arms. *JAMA* 2009; 301: 619-28
- 11 Gart MS, Souza JM, Dumanian GA. Targeted Muscle Reinnervation in the Upper Extremity Amputee: A Technical Roadmap. *J Hand Surg Am* 2015; 40: 1877-88
- 12 Cheesborough JE, Smith LH, Kuiken TA, Dumanian GA. Targeted muscle reinnervation and advanced prosthetic arms. *Semin Plast Surg* 2015; 29: 62-72
- 13 Yoshida K, Horch K. Selective stimulation of peripheral nerve fibers using dual intrafascicular electrodes. *IEEE Trans Biomed Eng* 1993; 40: 492-4
- 14 Polasek KH, Hoyen HA, Keith MW, Tyler DJ. Human nerve stimulation thresholds and selectivity using a multi-contact nerve cuff electrode. *IEEE Trans Neural Syst Rehabil Eng* 2007; 15: 76-82
- 15 Larsen JO, Thomsen M, Haugland M, Sinkjaer T. Degeneration and regeneration in rabbit peripheral nerve with long-term nerve cuff electrode implant: a stereological study of myelinated and unmyelinated axons. *Acta Neuropathol* 1998; 96: 365-78
- 16 Stieglitz T. Flexible biomedical microdevices with double-sided electrode arrangements for neural applications. *Sensors and Actuators a-Physical* 2001; 90: 203-211
- 17 Zheng XJ, Zhang, J., Chen, T., Chen, Z. Longitudinally implanted intrascicular electrodes for stimulating and recording fascicular physioelectrical signals in the sciatic nerve of rabbits. *Microsurgery* 2003; 23: 268-273
- 18 Micera S, Navarro X. Bidirectional interfaces with the peripheral nervous system. *Int Rev Neurobiol* 2009; 86: 23-38
- 19 Urbanek MG, Wei B, Egeland BM, Abidian MR, Kipke DR, Cederna PS. Microscale Electrode Implantation during Nerve Repair: Effects on Nerve Morphology, Electromyography, and Recovery of Muscle Contractile Function. *Plast. Reconstr. Surg* 2011; 128: 270e-278e
- 20 Lefurge T. Chronically implanted intrafascicular recording electrodes. *Annals of Biomedical Engineering* 1991; 19: 197-207