IMPROVED LONG-TERM RECORDING OF NERVE SIGNAL BY MODIFIED INTRAFASCICULAR ELECTRODES IN RABBITS

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Methods for long-term recording of peripheral nerve activity via intrafascicular electrodes have not been optimized. We compared the long-term functionality of custom-made 95%Pt/5%Ir intrafascicular electrodes containing a proximal spring-like structure to that of conventional straight electrodes. The modified electrode was implanted into the sciatic nerve fascicle of a random hind limb in 14 rabbits for 9 months. A conventional electrode was implanted in the opposite hind limb as a control. Orthodromic and antidromic nerve potentials were sampled and analyzed monthly. Latency, amplitude, and nerve conduction velocity of electrical signals were generally similar within the modified group and straight control group at different time intervals ($P > 0.05$). However, at the conclusion of the study period, the modified electrode group had a greater number of functioning electrodes ($P < 0.05$) and a greater total functioning electrode time ($P = 0.006$). Intrafascicular electrodes with a spring-like structure demonstrated superior potential for long-term electrophysiological monitoring over straight electrodes. © 2008 Wiley-Liss, Inc. Microsurgery 28:173–178, 2008.

Many different kinds of commercial upper limb prostheses[1–6] are available in the world, but the cosmetic and functional results are inconsistent and the rate of rejection of prostheses still remains unacceptably high (22–50%).[4,7–12] Use of neural signals for prosthesis control has great potential because this method harnesses nervous system plasticity and neural signals accurately reflect motor nerve commands from the CNS.[13] Neural signals are also highly stable, reproducible, and detectable.[14] Interfacing peripheral nerve signals with intrafascicular electrodes in the nerve stump has become an exciting focus of prosthesis control research.[14–23]

Straight intrafascicular electrodes have already been developed[24–29] to record peripheral nerve signals in animal models as well as clinical experiments.[18,20,23] Previously, implantation of straight longitudinal intrafascicular electrodes was guided with the aid of a 50 μm diameter tungsten needle, which was chemically bonded to the leading end of the intrafascicular electrode using cyanoacrylate adhesive. In this method, the needle and electrode are passed through the perineurium and then along within the endoneurium for 6–10 mm. The needle and electrode are then passed back out of the perineurium, allowing the experimenter to cut off the adhesive and remove the needle, leaving the electrode in proper placement and alignment.[18,20,23,29] However, the conventional straight longitudinal intrafascicular electrodes are difficult to keep in a fixed position, and can be easily dislodged during implantation surgery or electrophysiological experiment, making them a poor candidate for long-term implantations. Recent clinical studies with peripheral nerve signal controlled prostheses were performed using intrafascicular electrodes during a short time.[18,20,23] The use of long-term implantable intrafascicular electrodes to record peripheral nerve activity has not yet been developed.

We developed intrafascicular electrodes with a spring-like structure 8 mm from the proximal end. When implanted using direct microsurgical technique, we found that it stably and reproducibly recorded the instant electrical signal of the peripheral nerves after implantation[30–32] and demonstrated the feasibility of nerve signal-controlled artificial limbs for amputees.[14–17] In this study, we compared these two electrodes in rabbits to evaluate their long-term electrophysiological properties and to provide evidence for the future long-term study of these electrodes.

MATERIALS AND METHODS

Fourteen adult White New Zealand rabbits (3.0–3.5 kg) were used in this electrophysiology study. In each animal, one hind limb was randomly assigned to the straight electrode control group and the other to the modified implantation group ($n = 14$ per group). The experimental protocol was approved by the Fudan University Animal Care and Use Committee and all procedures were compliant with institutional guidelines for the care and use of laboratory animals. The rabbits had free access to food and water before and after the experiments, and were subjected to a 12 hour day/night cycle in a quiet environment.

Electromyelogram and nerve conduction velocity (NCV) measurements were made using a 4-channel

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Grant sponsor: National Natural Science Foundation of People's Republic of China; Grant number: 39930070.

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Received 10 August 2007; Accepted 16 October 2007

Published online 19 February 2008 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/micr.20475

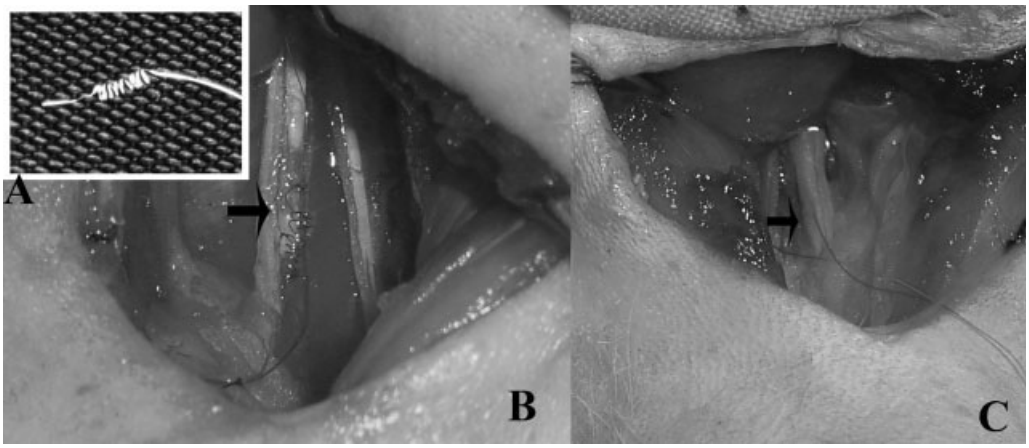

*Figure 1. Modified intrafascicular electrodes (→) with spring-like structure 8 mm from the proximal end (**A**, **B**) and straight intrafascicular electrode (**C**, →) were surgically implanted in the sciatic nerve.*

Haishenhao type 1 Paseidon NDI 500 (EMG) machine developed by the Navy Medical Institute of P.R. China.

Electrode Fabrication

All intrafascicular electrodes were fabricated from 80 mm of 60 μm 95% platinum/5% iridium alloy wire coated with 5 μm of Teflon. The modified electrodes had a spring-like structure fashioned 8 mm from the proximal end[31] (diameter 0.5 mm and 10 cycles) (Fig. 1A) in order to ensure firm fixation with micro-sutures. Approximately 1 mm of insulation at the proximal end of the electrodes was removed to prepare for implantation in the fascicle and 10 mm of insulation at the distal ends was removed for future attachment to the EMG instrument.[30,32] A similar 120-mm alloy wire was made as a reference electrode after removing the insulation at the proximal and distal ends in the same way.

Microsurgical Implantation of Intrafascicular Electrode

The animals were anesthetized with an intravenous injection of 3% sodium pentobarbital (3.5 mg/kg), and supplementary injections were given as needed to maintain anesthesia. Aseptic surgical procedures included shaving the hair from hind legs and dorsal feet, iodine sterilization of the skin, use of sterile drapes around the surgical field, and steam sterilization of all surgical equipment. Normal body temperature was maintained with a heating pad.

Animals were then fixed in the prone position. An incision was made along the thigh from the hip to the knee. The biceps femoris muscles were separated and retracted to expose the sciatic nerve over a length of 3–4 cm. The sciatic nerve was carefully isolated from the surrounding soft tissue for about 3 cm and kept moist with sterile saline. Under a 10× surgical microscope (Leica MC-1, Leica Microsystems, Switzerland), the largest fasciculus was chosen and ~2 mm of the epineurium was carefully teased open longitudinally with microsurgery equipment. Special attention was paid to ensure the perineurium remained intact. A nerve retractor was used to keep the nerves under moderate tension. Unlike other studies,[18,20,23,29] our electrodes were inserted only by microsurgical technique and were not attached with an extra hard tungsten needle in order to lessen the damage to the surrounding nerve tissue. Without separating the fasciculus, an intrafascicular electrode, held with a micro-needle-holder near the proximal end under a slight amount of tension, was carefully inserted under microscope at 60° oblique to the fasciculus until the tip pierced through the perineurium. The proximal end was threaded into the fasciculus for about 4 mm, and then advanced in a direction parallel to the nerve fasciculus until the whole length of the recording area was inside the fascicle. Control electrodes were sutured to the epineurium by using eight evenly-spaced sutures of 11-0 silk. In the modified group, electrodes were fixed at the point of the spring-like structure with the epineurium using three sutures of 11-0 silk and the rest of the electrode was secured with five sutures along the epineurium. Another wire was placed outside the epineurium of the sciatic nerve and parallel to the longitudinal direction of the nerve as a reference electrode, with its recording area aligned to that of the intrafascicular electrode. It was fixed to the epineurium by micro-sutures. (The intrafascicular electrodes implanted in the animal are shown in Figs. 1B and 1C).

Electrophysiologic Study

Recording orthodromic (type A) evoked potentials. Two concentric pin electrodes were fixed through a wood bar at a distance of 10 mm. After interfacing the ends of the electrodes to the EMG machine, two concentric pin stimulus

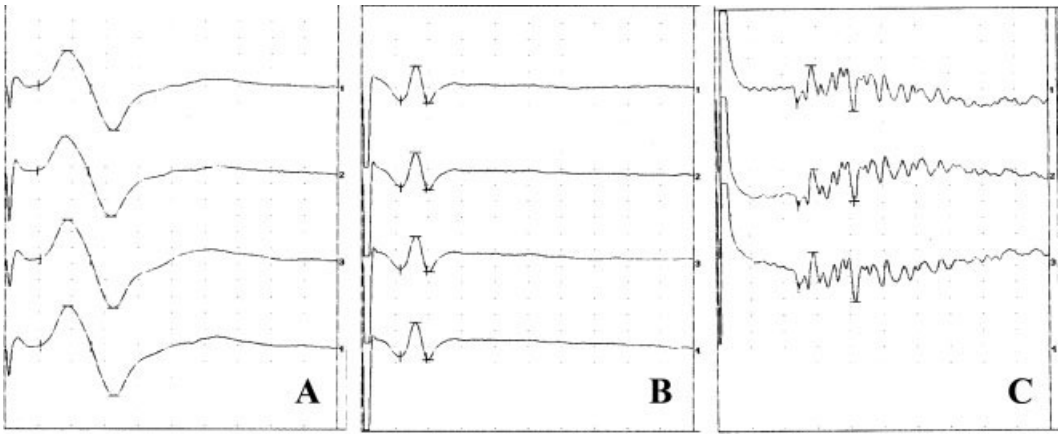

*Figure 2. Orthodromic evoked action potentials (**A**), antidromic evoked action potentials (**B**) and sensory nerve action potentials (**C**) were recorded by intrafascicular electrode in a rabbit sciatic nerve.*

electrodes were inserted at a point proximal to the intrafascicular electrode (which served as recording electrode) and the surrounding tissue was grounded. With the stimulus intensity of 9.6 mA, stimulus time of 2 ms, and sensitivity of 1 mV per division, the signal was recorded and the onset latency, interpeak amplitude and NCV were detected. Recording was repeated for four times and average latency, amplitude, and NCV were calculated.

Recording antidromic (type B) evoked potentials. Using two stimulus electrodes inserted at a point distal to the intrafascicular electrode (recording electrode), the antidromic nerve signal was recorded with the stimulus intensity of 10.7 mA, stimulus time of 2 ms, and sensitivity of 0.6 mV per division. The average latency, amplitude, and NCV were calculated with the same methods.

Recording somatosensory-evoked potentials. Stimulating with paired superficial ringed electrodes at the depilated first toe (stimulus intensity 22.0 mA, stimulus time 2 ms, sensitivity 3 μV per division, average overlap 80), sensory nerve action potentials (SNAP) were detected through the intrafascicular electrode. The average latency, amplitude, and NCV were calculated with the same methods.

The ends of electrodes attached with a silastic column were anchored to the subcutaneous tissue. After electrophysiological study was completed, the wound was irrigated and closed by layers. The surgical site was bound up with compression dressings. The rabbits fed as usual in the animal room after regaining consciousness. The sutures were removed about 10 days following the implantation.

The electrophysiological tests were repeated after 1 month via limit incision exploring the ends of electrodes and repeated every month until no signal was elicited for both hind limbs or for a total of 9 months. Isobody comparison was performed between the modified electrode and straight control electrode groups at different time intervals: the differences of type A, B, and C signals, the functional individual number and the functional period of intrafascicular electrodes were compared. The electric signals of type A, B, and C were compared, respectively, during different intervals after implantation of electrodes.

Statistical Analysis

Data are presented as mean ± standard deviation. Paired-sample *T*-test was used between control group and modified group. Univariate analysis was performed for parametric data with the use of the Student's *t*-test for continuous variables and the χ^2 test for categorical variables. Mann-Whitney test was used for nonparametric analysis of variance. Multivariate General Linear Model was used for advanced comparison of aggregate data to control for influencing factors. The mortality rate was analyzed by Fisher's exact test (crosstabs) and functional period was analyzed by a Kaplan-Meier test. Statistical significance was set at $P < 0.05$. All statistical analyses were performed with SPSS 12.0 for Windows software (SPSS, Chicago, IL).

RESULTS

Real-time electric nerve signaling was reliably detected by intrafascicular electrodes when electrophysiological tests were made. Related muscle groups contracted simultaneously distal to the implantation site when rectangular wave electric stimulation was applied. These signals were displayed on the EMG instrument (see Fig. 2). The signals of type A had the characteristics of the motor nerve signals with mono or double peak and broad shape of the peak. The signals of type B and C

Table 1. Comparison of Electric Signals After Implantation Between Modified Group and Control Group

	Control group	Modified group	*P* value
Type A amplitude (μV)	5,991 ± 5,059	4,356 ± 1,988	0.497
Type A latency (ms)	2.373 ± 0.813	2.337 ± 0.788	0.936
Type A NCT (m/s)	55.939 ± 12.987	50.364 ± 11.714	0.249
Type B amplitude (μV)	797.25 ± 712.19	1023.6 ± 1136.4	0.526
Type B latency (ms)	2.275 ± 1.044	2.002 ± 0.692	0.525
Type B NCV (m/s)	17.493 ± 8.882	22.202 ± 9.096	0.245
Type C amplitude (μV)	1.872 ± 2.822	5.201 ± 5.975	NA
Type C latency (ms)	4.356 ± 1.770	4.870 ± 0.660	NA
Type C NCT (m/s)	48.679 ± 17.037	42.238 ± 6.168	NA

NA, No comparison in type C was analyzed due to limit number in both groups.

had the characters of the sensory nerve: type B has mono sharp peak and type C has multiphase wave.

Postimplantation Instant Stability and Electrophysiologic Characteristics

All intrafascicular electrodes in the modified group had good intra- and post-operative stability and none of these electrodes were dislodged. Electric signal was elicited reliably by modified intrafascicular electrodes for all type A and B electrophysiological tests and 6 of 14 rabbits also had type C successfully elicited (Table 1). For the remaining 8 of 14 rabbits, type C signal could not be elicited, although stimulus intensity was increased and the surrounding muscles contracted at the same time.

For the control electrodes, 12 of 14 intrafascicular electrodes had good intra- and post-operative stability for type A and B signal. Muscle contraction by electric simulation during postimplantation instant electrophysiological test caused two electrodes to dislodge. Type C signals were successfully elicited in 4 of 12 rabbits (Table 1).

The amplitude, latency, and NVT of type A and B postimplantation instant signals were similar between control and modified electrodes ($P > 0.05$). No comparison in type C was analyzed due to a limiting number in both groups.

Long-Term Characters of Intrafascicular Electrodes

Type A and B signals were stably elicited by modified intrafascicular electrodes until the end of the electrophysiological experiment. No broken or separated electrodes were found in modified group. For the control electrodes, seven rabbits did not complete the electrophysiological study due to broken or dislodged electrodes. There were significant differences in the number of functional electrodes ($P < 0.05$) and electrode functional periods ($P = 0.006$) between the modified and control electrodes.

Long-Term Electrophysiologic Characteristics

The shape of the elicited signals were similar between control and modified group and the character of electric signals was generally similar among the two groups (all $P > 0.05$). The differences of their latent period, amplitude, and NCV within modified group and straight group at different intervals were not significant (all $P > 0.05$).

The waveform of electric signals in group A, B was generally stable during different period and the differences in the latent period and NCV were not significant ($P > 0.05$). The difference of amplitude was significantly decreased after 1 month ($P = 0.035$), but later recovered after 2 months ($P > 0.05$), and became higher than the instant signals after 6 months ($P = 0.012$).

DISCUSSION

Intrafascicular electrodes reliably and reproducibly capture instant peripheral motor nerve signals, making them suitable for a bionic prosthesis control.[14–17] This method is preferable to myoelectric methods because it allows for closed-loop feedback,[2] it circumvents the phenomenon of muscle fatigue,[1] and it does not limit control to one movement at a time.[33]

Our experiment demonstrated that our custom-made modified intrafascicular electrode can provide long-term monitoring of nerve activity. Instead of 90% Pt/10% Ir wire, we used a more flexible 95% Pt/5% Ir wire and manufactured a spring-like structure near the end. This structure secured against shearing or stretching forces imposed by surrounding soft tissues during limb movements[31] and protected the electrode from the dislodgements by intra-operation longitudinal traction or intra-stimulation muscle contraction, and from breakages by the activity of surrounding soft tissue seen in the straight control electrodes. This modified spring-like structure did not change the electrophysiological signature of the electrodes. Thus, the modified design increased the functional individual number of intrafascicular electrodes for long-term study.

Instead of an implantation guided with an extra tungsten needle which doubles the effective diameter of electrodes, makes another hole throughout the perineurium and epineurium, and creates a second exposure at the distal end the electrode,[18,20,23,28,29] we use direct microsurgical implantation. This technique decreased injury to

fascicles, avoided possible external interference, and provided relatively better insulation.[30–32,34]

Our experiment showed SNAP could be harvested by the intrafascicular electrodes. That SNAP could not be elicited in all the animals may be due to the selective conduction of the intrafascicular electrode. We only implanted the intrafascicular electrode in the largest fasciculus and it may not have contained axons involved in the specific pathway we stimulated. The relatively lower amplitude (5.021 μV in the modified group and 1.872 μV in the control group) of SNAP stimulus may not have excited the fasciculus with the intrafascicular electrode through contact conduction via insulated perineurium.

The nerve signal was reliably recorded by the intrafascicular electrode throughout the valid functional periods. The shape of waveform of type A and B signals was similar and the differences of the latent period and NCV were not significant during different periods ($P > 0.05$), which demonstrated the good performance of the custom-made intrafascicular electrode. The change of amplitude of the nerve signal reflects the reaction of surrounding tissue. Although more flexible material was chosen (95% Pt-5% Ir), it was still more rigid than the surrounding fascicle. The decreased amplitude after 1 month post-implantation may be due to nerve injury from slow micro-movements of the electrode between unmatched surrounding soft tissue and relatively more rigid intrafascicular electrode which start after the implantation, reach a peak after 1 month, and then abate by month 2 as tissue reactions fix the electrode in place. The body healing process from the injury of implantation and electric stimulation made the amplitude gradually recover and reach the normal level (higher than the signal immediately postimplantation) after 6 months. This preliminary exploration provides useful information for further establishment and development of the artificial limb controlled by nerve signals.[14–17]

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