

Appendix

CMAP

After exposure of the sciatic nerve proximal to the graft, a miniature bipolar stimulating electrode (Harvard Apparatus, Holliston, Massachusetts) was clamped to the exposed sciatic nerve. Ground and recording electrodes were placed in the adjacent subcutaneous tissue and the tibialis anterior muscle surface, respectively. The compound muscle action potential (CMAP) was measured with use of a Viking Quest Portable EMG (CareFusion, San Diego, California). With the stimulus duration set at 0.02 ms, the stimulus intensity was optimized to the minimum necessary to elicit a maximum CMAP signal. The maximal amplitude of the depolarization curve was then recorded. The contralateral, control side was tested in an identical fashion. The skin was reapproximated with suture on completion of CMAP testing until force measurements were conducted.

Maximum Isometric Tetanic Force

The optimal muscle preload (i.e., muscle fiber length) and nerve stimulus parameters were determined, and this was followed by recording of the resultant maximum muscle tetanic contraction. The tibialis anterior tendon insertion was released, and the muscle belly was freed from fascial connections while its neurovascular pedicle was preserved. The hindlimb was stabilized with two Kirschner wires (Pfizer Howmedica, Rutherford, New Jersey), one placed in the distal part of the femur and one placed in the ankle joint, with preservation of the neurovascular structures. The distal part of the tibialis anterior tendon was attached to a mini-load cell (MDB-2.5 lbs; Transducer Techniques, Temecula, California) by a custom clamp aligned to replicate the tendon's original anatomical orientation. The peroneal branch of the sciatic nerve was exposed and connected to a stimulator (Grass SD9; Grass Instrument Company, Quincy, Massachusetts) by a bipolar electrode (Harvard Apparatus) (Fig. 2). During force testing, body temperature was maintained at 37°C with a heating pad, and the muscle was kept moist with a heated (37°C) saline-solution drip. The optimal muscle fiber length (preload), stimulus intensity, and pulse duration were sequentially determined by a single electrical stimulus at 10 Hz. Three seconds of continuous electrical stimulus was used to optimize the stimulation frequency, and finally the maximal isometric tetanic force contraction was recorded, processing the force transducer signal with use of LabVIEW software (National Instruments, Austin, Texas). The contralateral, control side was measured in an identical fashion.

TABLE E-1 Summary of Results of Tests for All Groups*

| | | Group I | | Group II | | Group III | |
|---|--------------------------------------|--------------|--------------|--------------|--------------|------------------|---------------|
| | Repair type | Autograft | | Allograft | | Collagen Conduit | |
| | Sacrifice time (<i>wk</i>) | 12 | 16 | 12 | 16 | 12 | 16 |
| | Rat initial weight (<i>g</i>) | 272.5 ± 25.5 | 272.2 ± 27.7 | 277.2 ± 36.4 | 280.7 ± 30.7 | 258.7 ± 11.2 | 255.0 ± 12.0 |
| | Rat final weight (<i>g</i>) | 395.3 ± 19.8 | 405.7 ± 22.2 | 405 ± 36.6 | 418.9 ± 29.7 | 370.1 ± 29.0 | 379.7 ± 37.8 |
| | % weight gain | 46.0 ± 13.0 | 50.0 ± 12.1 | 47.2 ± 12.0 | 50.1 ± 14.5 | 43.1 ± 10.6 | 48.9 ± 12.8 |
| Tib. ant. muscle: CMAP (normalized to contralateral side) | Amplitude recovery (%) | 54.5 ± 63.0 | 70.5 ± 34.5 | 37.3 ± 15.4 | 39.4 ± 20.2 | 5.2 ± 7.2 | 18.8 ± 30.5 |
| | Tib. ant. recovery rate | 10/10 | 10/10 | 10/10 | 9/10 | 3/10 | 5/11 |
| | Number of animals tested | 10 | 10 | 10 | 10 | 10 | 11 |
| Maximal isometric tetanic tension (normalized to contralateral side) | Tib. ant. muscle force recovery (%) | 45.2 ± 15.0 | 65.5 ± 14.1 | 43.4 ± 18.0 | 36.3 ± 15.7 | 7.0 ± 9.2 | 12.1 ± 16.0 |
| | Tib. ant. recovery rate | 10/10 | 10/10 | 10/10 | 9/10 | 3/10 | 5/11 |
| | Number of animals tested | 10 | 10 | 10 | 10 | 10 | 11 |
| Tib. ant. muscle weight (normalized to contralateral side) | Tib. ant. muscle weight recovery (%) | 55.1 ± 7.1 | 64.1 ± 7.2 | 55.1 ± 4.8 | 50.4 ± 13.0 | 22.6 ± 8.8 | 28.9 ± 17.9 |
| | Number of animals tested | 10 | 11 | 10 | 10 | 10 | 11 |
| Maximum passive plantar flexion ankle angle | Ankle angle (<i>deg</i>) | 137.5 ± 14.0 | 142.2 ± 17.5 | 130.6 ± 20.8 | 120.5 ± 15.7 | 101.4 ± 11.5 | 97.6 ± 11.5 |
| | Number of animals tested | 10 | 11 | 10 | 10 | 10 | 11 |
| Histomorphometry: peroneal nerve (normalized to contralateral side) | Nerve area (%) | 91.8 ± 24.4 | 126.9 ± 56.3 | 114.6 ± 38.7 | 99.8 ± 44.9 | 71.4 ± 38.0 | 84.6 ± 42.0 |
| | Number of axons (%) | 153.5 ± 54.5 | 151.6 ± 71.5 | 157.9 ± 44.4 | 111.8 ± 69.2 | 39.0 ± 42.1 | 112.4 ± 130.5 |
| | Myelin fiber diameter (%) | 53.0 ± 6.6 | 82.5 ± 49.5 | 63.7 ± 31.2 | 82.0 ± 53.2 | 60.7 ± 23.9 | 87.5 ± 48.9 |
| | Axon diameter (%) | 44.9 ± 7.3 | 83.5 ± 71.1 | 61.2 ± 50.1 | 80.3 ± 79.1 | 50.4 ± 29.0 | 93.8 ± 84.0 |
| | Myelin thickness (%) | 70.3 ± 13.1 | 87.1 ± 23.0 | 74.9 ± 15.5 | 95.0 ± 18.8 | 84.9 ± 23.7 | 97.4 ± 21.2 |

| | | | | | | | |
|--|-----------------------------|------------|-------------|-------------|-------------|-------------|-------------|
| | G ratio (axon/fiber) (%) | 84.7 ± 7.4 | 92.2 ± 18.4 | 89.1 ± 17.6 | 85.7 ± 23.4 | 79.9 ± 14.1 | 92.3 ± 31.6 |
| | Number of animals tested | 10 | 11 | 10 | 10 | 10 | 11 |

*Muscle force, muscle weight, CMAP, and histomorphometric results are expressed as a percentage of the values on the contralateral side (mean ± standard deviation). The results of passive ankle angles are expressed as absolute values in degrees (mean ± standard deviation). tib. ant. = tibialis anterior.

TABLE E-2 Pearson Correlation Coefficient (r) Between Different Tests Measured at Sixteen Weeks After Surgery (N = 24)*

| | Number of Axons | Myelin Thickness | Axon Diameter | Fiber Diameter | Nerve Area | CMAP | Ankle Angle | Muscle Weight |
|------------------|-----------------|------------------|---------------|----------------|------------|-------|-------------|---------------|
| Muscle force | 0.32 | -0.32 | 0.11 | 0.09 | 0.42 | 0.80* | 0.77* | 0.89* |
| Muscle weight | 0.32 | -0.29 | 0.21 | 0.17 | 0.52 | 0.73* | 0.83* | |
| Ankle angle | 0.046 | -0.12 | 0.20 | 0.21 | 0.27 | 0.62* | | |
| CMAP | 0.29 | -0.43 | 0.13 | 0.07 | 0.31 | | | |
| Nerve area | 0.39 | 0.09 | 0.29 | 0.31 | | | | |
| Fiber diameter | -0.004 | 0.51 | 0.98* | | | | | |
| Axon diameter | 0.038 | 0.37 | | | | | | |
| Myelin thickness | -0.39 | | | | | | | |

*Asterisks indicate a coefficient of ≥ 0.6 .