INTRODUCTION: The current clinical repair method for the segmental peripheral nerve defect is an autogenous nerve grafting. However, the autogenous nerve grafting have some disadvantages, such as a necessity of donor nerve, a sensory loss at the donor site, and even formation of neuroma. In addition, it is not propitious for regeneration of nerve that regenerating axons across at the two coaptation sites. Therefore, we investigated a new repair method for the segmental peripheral nerve defect by lengthening nerve directly. Our previous studies have shown that the repair of the peripheral nerve defect with direct lengthening of nerve stumps, in which the nerve lengthening was under anesthesia, resulted in successful nerve regeneration, and more beneficial to recovery of nerve function compared with the autogenous nerve grafting in rats (1,2,3). For the clinical application, we have designed a new device, which was modification of the finger fixator BL 2000 of ME system Inc (patent pending), for gradual nerve lengthening without anesthesia, and investigated whether the repair of a larger nerve defect is feasible in the larger lab animal model.

METHODS: Fifteen adult Japanese white rabbits were used. A nerve segment of 20 mm in length was resected from the sciatic nerve under the anesthesia. In the nerve lengthening group (Fig. 1a), the proximal and distal nerve stumps were fixed to the ring with 4-0 nylon suture respectively. The traction sutures (3-0 polyester sutures) which attached to the ring were bounded to the external fixator for nerve distraction. At a point 1mm to the rings of both stumps, a marking suture (9-0 nylon) was placed in the epineurium. The both stumps were distracted via the traction sutures for the following day operation without the anesthesia. The distraction was performed at a rate of 1mm/day.

Experiment I: Histological evaluation of the lengthened nerve stumps
At 22 day after the initiation of nerve lengthening (n=6), the nerve was exposed under the anesthesia. The distance between the rings and the marking sutures as well as the overlapping length of both nerve stumps were measured with a vernier caliper. The lengthened nerve were harvested just at the rim of the rings, and immunostaining was performed to detect neurofilaments and s-100 proteins (n=3).

Experiment II: Evaluation of nerve regeneration
In the lengthening group, after the distraction of 22 days, the second operation was performed. Both stumps were refreshed and a direct the end-to-end anastomosis was performed. For the control, the autogenous nerve grafting procedure was performed in which the resected nerve segment of 20 mm length was implanted immediately in reverse direction (nerve grafting group, Fig. 1b). At 16 week after the first operation, nerve regeneration was evaluated in both groups (n=6, each group). Motor nerve conduction velocity (MCV) and wet weight of gastrocnemius muscle were evaluated. The data were expressed as a percentage of contralateral extension. A nerve segment was resected from the tibial nerve at 10 mm proximal to the entrance of the gastrocnemius muscle and a 1 mm transverse section was made. The mean number of myelinated fibers in each field (each field covered 0.07 mm² in area, ×400) and the mean axonal diameters were analyzed in three random fields, and a histogram of axonal diameter was constructed. Statistical analysis of Student t-test was used and a P value at 0.05 or less was considered for statistical significance.

RESULTS:

Experiment I: Histological evaluation of the lengthened nerve stump
There was no painful behavior observed at the time of lengthening without the anesthesia. At 22 day after lengthening, the distance between the ring and the marking suture increased from 1 mm to 9.7±2.6 mm in the proximal stumps, and 4.8±0.8 mm in the distal stump. The length of the overlapping part of the both stumps increased to 14.2±0.9 mm.

In the longitudinal sections of the lengthened nerve, the stained neurofilaments and S-100 proteins were observed throughout the proximal nerve segment reaching to the ring, while stained S-100 protein were observed throughout the distal nerve segment reaching to the ring (Fig. 2.).

Experiment II: Evaluation of regeneration
At 16 week, the MCV and muscle wet weight were significantly greater in the lengthening group than in the grafting group (P<0.05, **P<0.01, Fig. 3. 4.). Histologically, the mean number of myelinated fibers in each field was significantly greater in the lengthening group than in the grafting group (**P<0.01, Fig. 5.). The mean axonal diameter was significantly greater in the lengthening group than in the grafting group (**P<0.01, Fig. 6.). The histogram at 16 week also showed that there were more axons with a large diameter in the lengthening group than in the grafting group (Fig. 7.).

REFERENCES: