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# Animal Model for the Evaluation of Repair of Injured Inferior Alveolar Nerve with Nerve Growth Factor

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**Purpose:** The inferior alveolar nerve (IAN) can be damaged as a result of minor oral surgical procedure such as third molar extraction or implant placement. Repair of the injured IAN involves difficulty of access, and research studies are limited to elucidating the process of regeneration by surgical methods. This study sought to establish the rabbit animal model to apply polymeric membrane functionalized with nerve growth factor after a crush lesion for the evaluation of nerve regeneration using the electrophysiologic method.

**Materials and Methods:** The IAN of 2 adult male New Zealand white rabbits (4 nerves) were exposed bilaterally, and crush injury rendered by jeweler's forceps was applied. Nerve conduction velocity was examined electrophysiologically using electromyography before, after, and 4 weeks after the crush injury. To evaluate the regeneration, the pattern of action potential of IAN was recorded, and the characteristics of neurons were histologically observed.

**Result:** After the crush injury, afferent activity decreased in the injured group. Electromyography could not be recorded after four weeks because tissues surrounding the injured nerve collapsed. Decrease in the mean number of axons was observed in the injured part with membrane.

**Conclusion:** Despite the limited result, the present animal model study may provide a possible way to research on the methods of enhancing the recovery of nerve injuries in clinical situations. For clinically widespread acceptance, however, it should gain more consecutive and scientific evidences.

**Key Words:** Mandibular inferior alveolar nerve; Models, animal; Nerve growth factor; Nerve regeneration

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## Introduction

Since nerves are frequently damaged nowadays due to the extraction of the third molar and surgeries involving implants and orthognathic surgery, and the resulting sequela increases, the meaning of nerve is perceived as more than simple anatomical structure unlike before. In addition, since there are more cases of experiencing paresthesia after surgery, interest in therapy designed to recover the nerve function after injury has also increased. In most cases, patients experience paresthesia after minor oral surgery because traumatic damage occurred on the inferior alveolar nerve (IAN). The damaged IAN causes patients to feel pains due to minor stimulation or to suffer considerable pains due to the improper delivery of sense; they are affected mentally, causing hindrance to daily activities in serious cases[1].

Since there have been no clear solutions presented to operators except drug therapy as a non-surgical method[2], many dentists are at a loss as to what to do if the symptoms of patients are not improved by drug. IAN damage is considered to be a medical fault that might lead to lawsuits. Therefore, studying the method of regenerating nerve carries extremely high clinical meaning.

Many research studies carried out on the peripheral nerve disclosed that Wallerian denaturation has occurred in the efferent division of the damaged part following traumatic damage, and that axon grows from the proximal area, leading to the regeneration of functions[3-6]. Various kinds of studies including histologic and immunologic studies as well as those on the method of treating the damaged part have been conducted. Nowadays, there are many molecular biological and tissue engineering approaches such as stem cell or cytokine[4,7]. Studies related to IAN have been consistently carried out as well[1,8,9]; in most cases, they were related to the nerve graft through microsurgery and nerve regeneration using histologic carrier, focusing on function recovery. According to Gregg[1], the symptoms may be improved for patients with damaged IAN by surgically decompressing nerve tissue through a surgical method. He prescribed delayed surgical method wherein patients wait for natural healing for 3 months prior to performing surgery. Note, however, that he could not present sufficient scientific grounds related to this method. Eckardt et al.[8] announced that they had obtained meaningful results in regenerating nerve even 12 months after damage by conducting research wherein tibial nerves were grafted and connected to the IAN cut from rabbits.

Many research studies related to the damage and regeneration of IAN to date presented affirmative results. Note, however, that there were also limitations, i.e., it is unreasonable to apply the animal test results as they are to actual clinical trials. In particular, it is clinically difficult to open the mandible for nerve graft and transplant tibial nerve or connect the cut nerve using microsurgery.

Based on recent research studies, Savignat et al.[9] reported, based on rat tests, that initial nerve regeneration was improved using the membrane wherein the nerve growth factor (NGF) was adsorbed into the damaged mental nerve. Since this method is simple compared with the existing nerve connecting method, and it showed good results in animal tests, it is possible to use for clinical purposes. To verify clinical availability, more detailed research should be conducted in various aspects regarding the impact of the NGF on nerve regeneration and the method for the delivery system that can stably supply nerves. In order for such research to verify the effects of clinical application based on reliable scientific grounds, an effective method of verifying the regeneration of nerve tissue is needed. Nonetheless, there are practically no research studies on the same animal experimental models that can be applied

to repeated tests. Furthermore, even the animal experimental models announced to date are limited to histologic research[10]. Therefore, this study attempted to develop animal models capable of conducting electrophysiologic tests that can verify IAN damage and recovery of functions using the NGF and polymeric membrane.

## Materials and Methods

### *1. Experimental Animal*

As experimental animals, 2 healthy male New Zealand rabbits weighing approximately 3.0 kg (New Zealand white rabbits; Orient Bio Inc., Seongnam, Korea) were used. They were raised by allowing the self-feeding of solid feeds designed for animals and disinfected feeds and water. This test was conducted after undergoing examination by the Animal Experiment Ethics Committee of Bucheon St. Mary's Hospital, The Catholic University (BSM12-001).

### *2. Surgical Procedure*

For general anesthesia, 0.2 mg/kg of tiletamine HCl and zolazepam HCl (Zoletil; Virbac, Carros, France) and 10 mg/kg of xylazine (Rumpun; Bayer, Seoul, Korea) was intramuscularly injected into the thigh of the rabbits. Body hair was removed

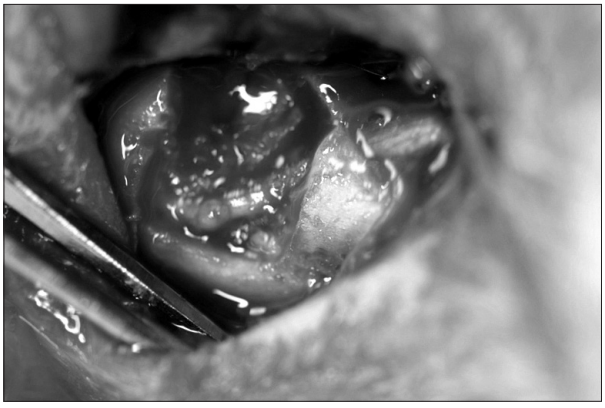


**Fig. 1.** Opening the bony window to expose and access the inferior alveolar nerve.

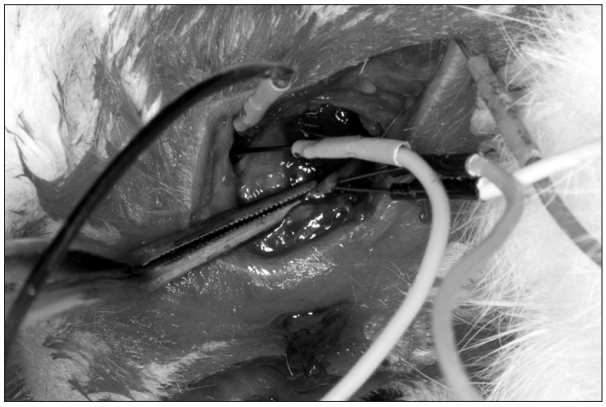


**Fig. 3.** Monopolar electrodes were inserted again at the proximal portion of the injured inferior alveolar nerve, mental nerve, and masseter muscles right after the crushing injury of the inferior alveolar nerve.

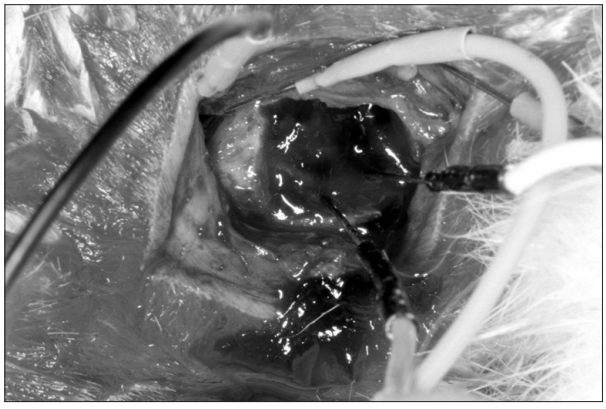


**Fig. 2.** Monopolar electrodes were inserted into the proximal portion of the inferior alveolar nerve, mental nerve, and adjacent masseter muscles before inducing nerve damage.

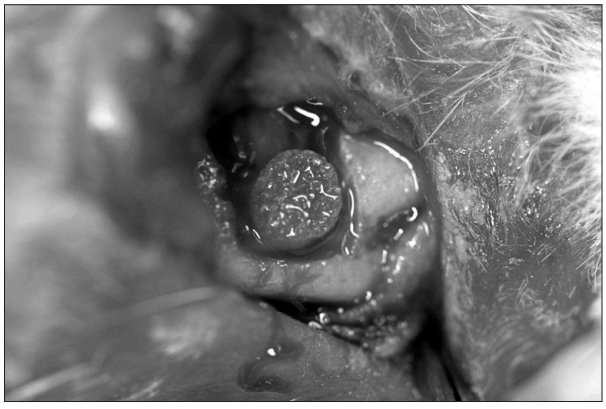
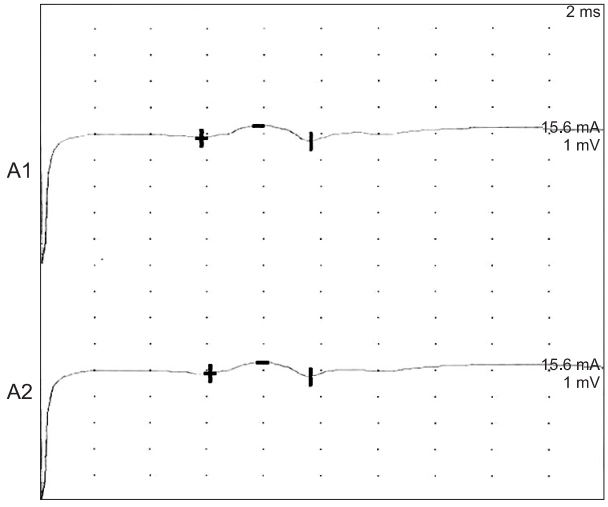


**Fig. 4.** Nerve growth factor-impregnated polylactic-co-polyglycolic acid membrane was applied to the injured inferior alveolar nerve.

from the rabbits' anesthetized lower jaw, which was fixed on the operating table after disinfecting it with betadine. For stanching purposes during surgery, 2% lidocaine HCl (Yuhan, Seoul, Korea) containing 1 : 100,000 epinephrine was injected into the operated area. To prevent infection, 5 mg/kg of animal gentamycin (Gentacin; Green Cross, Yongin, Korea) was intramuscularly injected into the thigh. The lower jaw of the rabbits was horizontally incised, and the muscle and periosteal were delaminated at the minimum level. The mental nerve exiting from the anterior premolar through the mental foramen was identified and carefully pulled, creating a 2×1 cm bony window on the posterior mental foramen to expose the IAN (Fig. 1).

### *3. Electrophysiologic Examination*

To check the loss of function after the IAN was injured, electrophysiologic tests were conducted prior to injury and immediately after applying compressive damage with 40 N/cm$^2$ force using jeweler's forceps[11]. First, 2 monopolar needle electrodes were located on the mesial segment and mental nerve of the expected damage area exposed using the mesial segment of the expected damage area of the IAN--which was exposed using the aforesaid method--and 1 monopolar needle electrode on the masseter muscle. For the nerve stimulation method, Viking IV (Nicolet Biomedical, Madison, WI, USA) was used, and compound action potentials were recorded from the distal segment while stimulating nerve from the mental nerve as the distal segment of the IAN damage

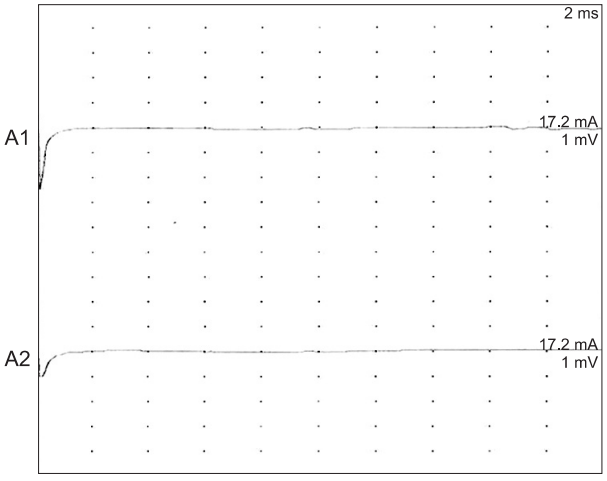



Recording site: mandibular

| Stimulus site | Lat1 (ms) | Dur (ms) | Amp (mV) | Area (mVms) |
|---|---|---|---|---|
| **A1: mandibular** | 5.7 U 8.4 | 3.8 | 0.5 | 1.0 |
| A2: mandibular | 6.1 U 7.5 | 3.5 | 0.5 | 1.0 |

| Segment | Diff (ms) |
|---|---|
| Mandibular-mandibular | 0.3 U 1.8 |

**Fig. 5.** Normal electrophysiologic action potential of inferior alveolar nerve before the injury. Lat1: latency 1 second, Dur: duration, Amp: amplitude, Diff: difference.




Recording site: mandibular

| Stimulus site | Lat1 (ms) | Dur (ms) | Amp (mV) | Area (mVms) |
|---|---|---|---|---|
| A1: mandibular | - | - | - | - |
| **A2: mandibular** | - | - | - | - |

| Segment | Diff (ms) |
|---|---|
| Mandibular-mandibular | - |

**Fig. 6.** Electrophysiologic action potential of inferior alveolar nerve after the crushing injury. Lat1: latency 1 second, Dur: duration, Amp: amplitude, Diff: difference.

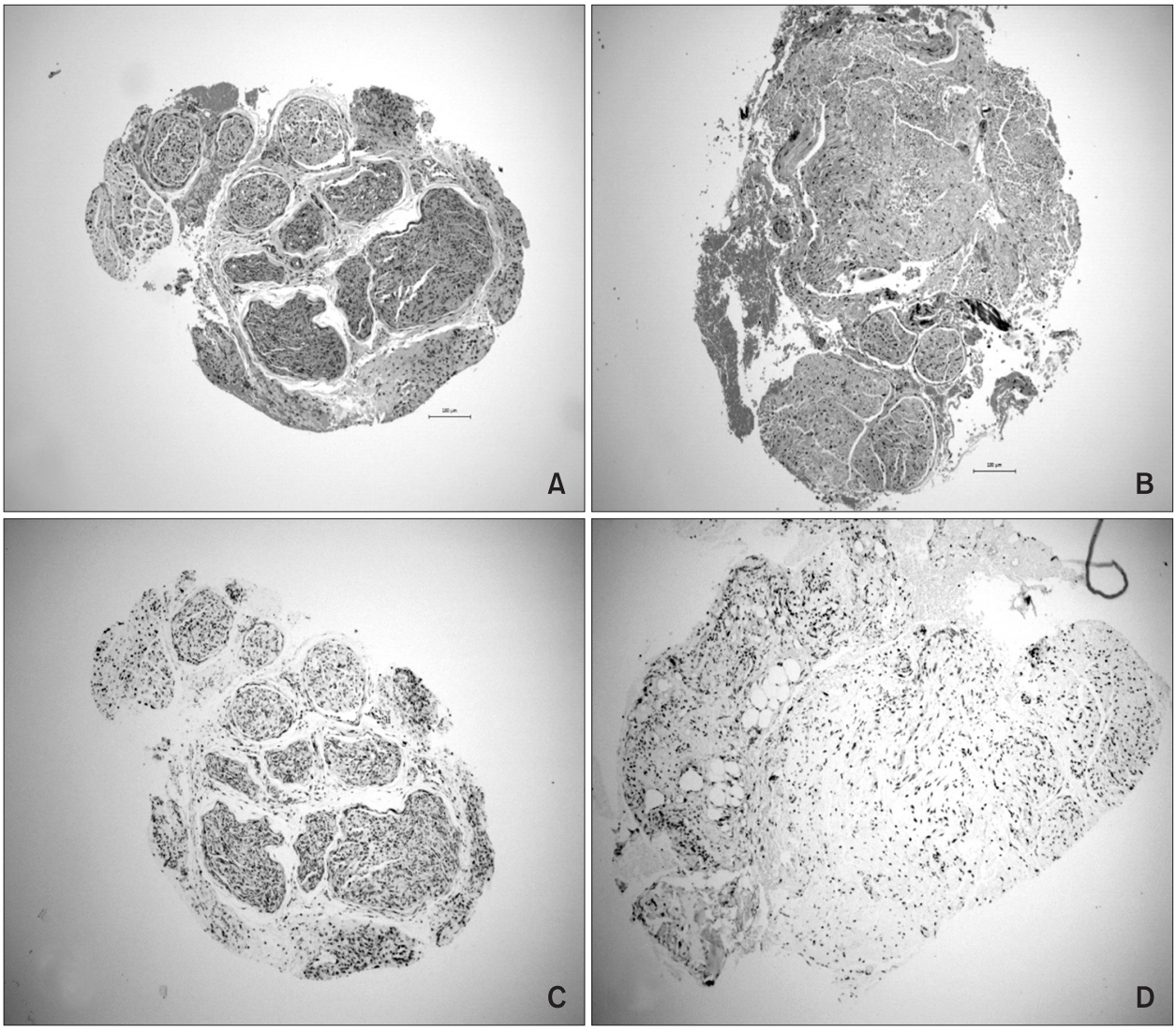



**Fig. 7.** Microscopic view of the histologic section. The distal nerve end 4 weeks after the crushing injury and nerve growth factor application (A, C) shows thicker and better-formed myelin than the control group (B, D). Note, however, that the outlines of axons are irregular in both groups. (A, B) H&E staining, ×100; (C, D) toluidine blue staining, ×100.

using 17.2 mA persistent current electricity for 0.1 ms (Figs. 2, 3).

Upon completion of the electrophysiologic tests, 25 μl of NGF with 3 mg/ml concentration was adsorbed into the polylactic-co-polyglycolic acid membrane on the other side of the left part; it was divided into the experimental side that covered the damaged IAN and into the control side where the NGF was not applied. The operated area was sutured by layer using absorbable suture (Fig. 4).

### *4. Histologic Examination*

Four weeks after the IAN was injured, the animals were sacrificed by intramuscularly injecting an overdose of anesthetic; the same area was opened, and approximately 1 cm of the IAN including the damaged area was taken. The amputated tissue was fixed in 10% neutral formaldehyde and embedded in paraffin to make a 4 μm-thick segment. Each tissue segment was stained with H&E and toluidine blue and was observed through the optical microscope.

## Result

### *1. Electrophysiologic Examination*

During the process of electrophysiologic examination, the normal IAN prior to injury showed 3.8 and 3.5 ms duration and 0.5 and 0.5 mV after the 8.4 and 7.5 ms latency, respectively; note, however, that there were no reactions after being injured. In other words, the potential difference, activated more than the normal nerve tissue, significantly disappeared (Figs. 5, 6).

### *2. Histologic Examination*

In the biopsy carried out after sacrificing the animals in each group 4 weeks after the IAN was injured, the axon of the nerve tissue that had applied NGF carried chick myelin compared with the control group, showing the conventional appearance of oval shape. Nonetheless, the shape of axon was irregular in both experimental group and control group (Fig. 7).

## Discussion

The biggest complication after oral surgery is sensory disorder caused by nerve damage. The ratio generating traumatic damage to the peripheral nerve after treatment is not that high (0.5% to 2%). In most cases, even if sensory disorder is induced once, spontaneous recovery is possible within 6 months, and such sensory disorder rarely becomes permanent[2,12]. Nonetheless, patients who experience sensory disorder complain of various abnormal sensations or pain in relation to pronunciation, taking meals, shaving, or tooth brushing[2].

Research studies that histologically describe IAN injury have been carried out for a long time now; nowadays, research is conducted at the molecular levels[4,5,13]. When nerve is damaged, calcium ion-dependent axon denaturation is triggered on the distal segment of the damaged peripheral nerve, and Schwann cell secretes cytokine while myelin is decomposed. Approximately 1 week thereafter, the resident macrophage is proliferated, removing the axon and myelin debris that cause hindrance to regeneration; after the Schwann cell migrates, Wallerian denaturation in preparation for axon regeneration is triggered. When the receptor (NgR) on the cell surface and the signaling transmitter are combined with the myelin-associated glycoprotein of the myelin surface activating RhoA, GTPase as the macrophage is pushed to outside the basal lamina. After that, the damaged axon forms the growth cone reacting to the nerotrophic factor, matrix forming precursor, or metabolitic toward the direction of the basal lamina tube and regenerates Bungner bands.

The NGF or nerotrophic factor is secreted from the Schwann cell[14]; since the level of secretion is insufficient or inconsistent during the period of 4 to 8 weeks after injury, however, healing may be accelerated if the NGF is supplied from the outside[9,15]. Ultimately, the problem to be solved is the method of supplying an appropriate dose of NGF on a timely basis. Furthermore, in selecting the supplying method, appropriate measures should be considered to ensure that the externally supplied NGF retains activity without being biodegraded. Wang et al.[14] directly injected NGF many times into the lower jaw of the rabbit, which was sutured after undergoing distraction osteogenesis, to attempt nerve regeneration. This method is simple but carries demerits, e.g., it might leave scars after numerous injections, and it is impossible to verify whether an appropriate dose of NGF is being applied for an appropriate duration to the damaged part. Savignat et al.[9,15] conducted experiments wherein the NGF were adsorbed into the polymeric membrane for application to the damaged nerve. This method, too, cannot tell the secretion ratio of the NGF or the accurate amount, but they concluded that small doses continuously administered for a month accelerate nerve regeneration. They proved

experimentally that using polymeric membrane as the delivery system of NGF is effective in retaining the activity of the NGF and in aiding in nerve regeneration. Considering the fact that the attempt made to apply the NGF featured weak myelin denaturation level in the NGF application group, the results of experiments conducted in this study were meaningful.

Methods of clinically treating the injured nerve as described above include microsuture and autogenous nerve graft. The microsuture uses methods of suturing the epineurium and the nerve fascicles. Suturing nerve fascicles cannot be regarded as superior, and such may leave larger scars[13]. Most research theses used epineurium suture. In nerve graft, autogenous nerve or homogenous nerve is grafted; since the vascularization of the receptor is an important success factor, nerve 10% to 20% longer than the needed length must be transplanted. The nerve graft should carry the donor site; the possible mismatch between the donor site and the receptor, sensory deterioration of the donor site, and scars are pointed out as other demerits. Moreover, homogenous nerve graft is not used often due to immunoreactions[13,16,17]. Surgery performed without pulling the nerve is reported to lead to better prognosis of the autogenous nerve graft than microsuture[13].

In comparative studies of nerve restoration of the microsuture and laser suture, Curtis et al.[18] proved experimentally that laser joining showed neuron restoration similar to that of microsuture. Compared with microsuture, laser joining reduces time and decreases hindrance to recovery associated with bone reactions. It is said that, even though large cell reaction is a convenient method for operators, there are problems of thermal damage and intrabone hemorrhage.

In addition to this, they reported that nerve general experiments were attempted using membrane or tube. Entubulation was performed using autogenous vein or artificial tube, with a certain level of success shown as a method of promoting the restoration of damaged nerve. Nonetheless, autogenous vein graft entubulation had demerits, i.e., the grafted vein was destroyed due to insufficient strength, and the results of the animal experiment and clinical application were not the same[16,17,19]. According to Miloro and Macy[16], even though there was no statistically significant difference in experiments conducted to compare the method of entubulating the rabbit's amputated nerve with the expanded polytetrafluoroethylene (e-PTFE) membrane with autogenous nerve graft, the number of nerve fascicles and diameter of axon increased when e-PTFE was used. Still, they asserted that, even though nerve regeneration using polymeric membrane or e-PTFE membrane showed good results in general experiments, the recovery of sensory nerve was insufficient to apply it clinically to the trigeminal nerve. Even though many conventional methods for nerve restoration were presented, they showed limitations in their application to the clinical situation, e.g., surgery takes a long time or inconsistent results are shown after surgery; scientific grounds are also lacking[1]. Accordingly, the polymeric membrane into which the NGF was adsorbed reduces the surgery time without losing biomechanics. Therefore, it is a therapy that has significantly improved clinically in terms of regenerating the damaged nerve and restoring senses[9]. Nonetheless, there should be sufficient preclinical basis for this method to be used widely in a clinical setting. The same results should be obtained from repeated multiple experiments, and there should be no significant difference in animal experiments and clinical application. In connection with this, this study sought to establish animal experimental models through preliminary research.

This study attempted to conduct neurophysiologic tests using histologic tests and electrostimulation to establish experimental animal models regarding

the intraosseous restoration of the damaged IAN. Since it was impossible to determine whether the nerve tissue was functionally restored based on the histologic shape alone, electrostimulation tests were conducted from the neurophysiologic viewpoint. As an experiment on the functional recovery of nerve after injury, perception tests may be performed; note, however, that erroneous results such as the reflex reactions of animals may appear[10]. In the case of ordinary electrostimulation tests, the degree of nerve recovery was indirectly checked by verifying whether the depolarization of nerve was delivered through electromyography (EMG). Nonetheless, this study checked the recovery levels by directly inserting electrode into the nerve. The electrophysiologic tests carried out based on direct electrostimulation were extremely important in establishing experimental animal models to be used in the future. When an attempt was made to test nerve conduction prior to and immediately after the injury and to conduct tests again 4 weeks thereafter, however, tests could not be completed because the tissue has undergone fibrotic degeneration, making it impossible to insert EMG electrode.

## Conclusion

In conclusion, the limitation of this study as preliminary tests was that statistical analysis was impossible. Since the principal goal of this study was to establish experimental animal models wherein the impacts of the histologic and functional restoration of the nerve carried by the NGF and delivery system can be continuously observed in future studies, this experiment lacked samples from which statistics could be obtained. In subsequent studies to be carried out in the future, there is a need to make preparations to enable electrophysiologic tests on a living body even after a specified period and arrange sufficient samples that can carry statistical meaning to derive test results that can be accepted scientifically.

## References


1. Gregg JM. Surgical management of inferior alveolar nerve injuries (Part II): the case for delayed management. J Oral Maxillofac Surg. 1995; 53: 1330-3.
2. Renton T, Yilmaz Z. Managing iatrogenic trigeminal nerve injury: a case series and review of the literature. Int J Oral Maxillofac Surg. 2012; 41: 629-37.
3. Vougioukas VI, Siebert H, Heinecke K, Brück W. Effects of the immunomodulator linomide on macrophage migration and myelin phagocytic activity in peripheral nerve trauma: an experimental study. J Neurotrauma. 2001; 18: 555-62.
4. Gaudet AD, Popovich PG, Ramer MS. Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. J Neuroinflammation. 2011; 8: 110.
5. Brück W. The role of macrophages in Wallerian degeneration. Brain Pathol. 1997; 7: 741-52.
6. Bray GM, Aguayo AJ. Regeneration of peripheral unmyelinated nerves. Fate of the axonal sprouts which develop after injury. J Anat. 1974; 117: 517-29.
7. Terenghi G, Wiberg M, Kingham PJ. Chapter 21: use of stem cells for improving nerve regeneration. Int Rev Neurobiol. 2009; 87: 393-403.
8. Eckardt A, Meier K, Hausamen JE. Histomorphometric results after late microsurgical nerve grafting of the inferior alveolar nerve of the rabbit. Int J Oral Maxillofac Surg. 1990; 19: 312-4.
9. Savignat M, Vodouhe C, Ackermann A, Haikel Y, Lavalle P, Libersa P. Evaluation of early nerve regeneration using a polymeric membrane functionalized with nerve growth factor (NGF) after a crush lesion of the rat mental nerve. J Oral Maxillofac Surg. 2008; 66: 711-7.
10. Curtis NJ, Owen E, Walker DM, Zoellner H. Comparison of microsuture, interpositional nerve graft, and laser solder weld repair of the rat inferior alveolar nerve. J Oral Maxillofac Surg. 2011; 69: e246-55.

11. Bridge PM, Ball DJ, Mackinnon SE, Nakao Y, Brandt K, Hunter DA, Hertl C. Nerve crush injuries--a model for axonotmesis. Exp Neurol. 1994; 127: 284-90.
12. Krafft TC, Hickel R. Clinical investigation into the incidence of direct damage to the lingual nerve caused by local anaesthesia. J Craniomaxillofac Surg. 1994; 22: 294-6.
13. Lee SK, Wolfe SW. Peripheral nerve injury and repair. J Am Acad Orthop Surg. 2000; 8: 243-52.
14. Wang L, Zhao Y, Cheng X, Yang Y, Liu G, Ma Q, Shang H, Tian L, Lei D. Effects of locally applied nerve growth factor to the inferior alveolar nerve histology in a rabbit model of mandibular distraction osteogenesis. Int J Oral Maxillofac Surg. 2009; 38: 64-9.
15. Savignat M, De-Doncker L, Vodouhe C, Garza JM, Lavalle P, Libersa P. Rat nerve regeneration with the use of a polymeric membrane loaded with NGF. J Dent Res. 2007; 86: 1051-6.
16. Miloro M, Macy JM. Expanded polytetrafluoroethylene entubulation of the rabbit inferior alveolar nerve. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000; 89: 292-8.
17. Jones RH. Repair of the trigeminal nerve: a review. Aust Dent J. 2010; 55: 112-9.
18. Curtis NJ, Lauto A, Trickett R, Owen E, Walker DM. Preliminary study of microsurgical repairs of the inferior alveolar nerve in rats using primary suturing and laser weld techniques. Int J Oral Maxillofac Surg. 1998; 27: 476-81.
19. Dodson TB, Kaban LB. Recommendations for management of trigeminal nerve defects based on a critical appraisal of the literature. J Oral Maxillofac Surg. 1997; 55: 1380-6.