

# Investigation of Three Drugs With Neuroregenerative Effects on Inferior Alveolar Nerve Injury in Rats

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## Original Article

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# Abstract

## Purpose

Nerve injuries formed during oral and maxillofacial surgical operations occur usually by crush or tension-type damages rather than incisions or ruptures. Such nerve injuries can be treated by providing regeneration with pharmacological agents without surgical intervention. The aim of this study was to determine and compare the effects of dexamethasone, B vitamin complex, and nerve growth factor on peripheral nerve regeneration with a local application on the inferior alveolar nerve.

## Methods

Twenty-four Wistar Albino rats weighing 220-250 grams were used in the study. Experimental animals were divided into 4 groups. According to the groups, animals were treated with saline, dexamethasone, vitamin B complex and nerve growth factor. All animals were sacrificed on the 21st day and inferior alveolar nerves were resected for microscopic examination.

## Results

The integrity and irregularities of myelin sheaths and vacuole formation were evaluated under a light microscope. Statistical analysis was performed with SPSS version 17.0. When histopathological findings were evaluated, it was observed that dexamethasone and vitamin B application triggered regeneration. However, regeneration in the group treated with nerve growth factor was much more obvious.

## Conclusions

Local drug applications are beneficial for regeneration in inferior alveolar nerve injury. Considering their success, studies are needed to place growth factors in clinical practice.

## Introduction

Injuries to peripheral nerves in the jaw and face have been a part of life since the existence of humanity. However, understanding and treating the effects of nerve injuries have not been possible for centuries because of the misinformation about the primary regions of sensory perception in ancient times. Accurate information about the nerve mechanism was obtained through investigations after the world wars. The destructive effects of these injuries began to attract attention especially in the last three centuries; as a consequence, studies were initiated to find effective therapies based on a scientific foundation (Miloro 2013).

Peripheral nerves are the basic connections among the body, brain, and spinal cord. Peripheral axons are found in almost every area of the body, including muscles, connective tissue, skin, and meninges surrounding the brain. The peripheral nervous system separates itself from the central nervous system by consisting of only neurons located in the brain and medulla spinalis. One of these distinctions is that

peripheral nerve cells are quite different in terms of their response to injuries and diseases compared to the cells that react to them in the central nervous system. In the neuroscience literature, peripheral nervous system cells are emphasized as neurons that can demonstrate regeneration of axons in response to damage, unlike the central nervous system cells. The presence of axons that can demonstrate regeneration in neuropathies is an important fact. However, this regeneration is rather slow and may never occur if the distances to the target tissues are long (Zochodne 2008).

Nerve injuries formed during oral and maxillofacial surgical operations occur usually by crush or tension-type damages rather than incisions or ruptures (Kalender et al. 2014). Considering this type of injury, a successful functional recovery can be expected with spontaneous regeneration that may occur in the distal nerve stump (SEDDON 1943; Sunderland 1990; Kalender et al. 2014). Such nerve injuries can be treated with pharmacological agents without surgical intervention. The aim of medical treatment is to restore the function of the injured nerves, to shorten the period of regeneration, and to improve the life quality of patients (Cornwall and Radomisli 2000; Er et al. 2016).

Oral and maxillofacial surgeries that are performed especially in the lower jaw including impacted third molar surgery, dental implant applications, cyst or tumor operations, orthognathic surgery, pre-prosthetic surgery, arthroscopic surgery, salivary gland surgery, operations in maxillofacial trauma, and injection injuries can damage the inferior alveolar nerve, mental nerve or lingual nerve by crushing or compressing it (Cornwall and Radomisli 2000; Er et al. 2016; KÜÇÜKKURT et al. 2018). The total percentage of the inferior alveolar nerve and lingual nerve injuries varies between 0.5% and 5% after the extraction of the lower third molar (Swanson 1991; Valmaseda-Castellón et al. 2001; Kanagasabapathy and BrigitEapen 2014). The percentage of lingual nerve injuries has a lower frequency compared to inferior alveolar nerve injuries, with 0.02% to 1% (Renton and McGurk 2001; Kanagasabapathy and BrigitEapen 2014).

As a result of these damages, complaints such as permanent anesthesia, dysesthesia, pain, paresthesia (burning, stinging, numbness or tingling sensation) related to the affected region can be observed (Seo et al. 2018; KÜÇÜKKURT et al. 2018). Generally, spontaneous recovery is observed in most of the patients within 6-8 weeks, but the sensory loss may persist for 6 months or longer, and permanent neuropathic disorders may occur in such cases (Smith and Lung; Pogrel and Kaban 1993; KÜÇÜKKURT et al. 2018).

There are current studies on drugs that are thought to be successful in treating peripheral nerve injuries. However, the studies on the inferior alveolar, mental and lingual nerves are small in number. Current clinical approaches are mostly on systemic applications. The aim of this study was to determine and compare the effects of dexamethasone, B vitamin complex and nerve growth factor (NGF) on peripheral nerve regeneration with a local application on the inferior alveolar nerve.

## Methods

All animal experiments in this study were conducted according to the Guidelines for Istanbul University Laboratory Animals Local Ethics Committee. A total of 24 specific pathogen-free, male, Wistar rats (220 ±

30 grams) were purchased from the Department of Laboratory Animals Science, Aziz Sancar Institute of Experimental Medicine at İstanbul University.

Wistar Albino rats were divided into four groups of six animals each:

Group 1 was administered saline following the crush-type injury in the inferior alveolar nerve as the control group.

Group 2 was administered dexamethasone following the crush-type injury in the inferior alveolar nerve.

Group 3 was administered vitamin B complex following the crush-type injury in the inferior alveolar nerve.

Group 4 was administered NGF following the crush-type injury in the inferior alveolar nerve.

Ketamine Hydrochloride 60mg/kg and Xylazine Hydrochloride 5mg/kg were administered to 24 Wistar albino rats intraperitoneally. After the administration of anesthesia, the operation site was shaved and disinfected with povidone iodine. 1 ml subcutaneous extraoral and 1 ml submucosal intraoral local anesthetic were applied for each side in accordance with asepsis and antisepsis rules. Using sterile surgical instruments, a 1-cm incision was performed at the lower border of the mandible and the full-thickness flaps were elevated from both sides of the jaws. The nerves were exposed (Figure 1) by means of dissection and were crushed by means of the Mosquito forceps for 60 seconds (Figure 2). These regions were treated only with saline in 6 rats; with dexamethasone in 6 rats; with B vitamin complex in 6 rats, and with NGF in 6 rats. The operation site was closed primarily by resorbable suture material. The region was covered with a patch. After the surgery, ampicillin 6 mg/kg was administered subcutaneously and tramadol 2 mg/kg was administered orally. Ampicillin was given in order to prevent postoperative infection and tramadol was given to provide postoperative analgesia.

All animals were sacrificed for microscopic examination by intraperitoneal administration of sodium pentothal 135 mg/kg on the day 21. Biopsy specimens were taken from the nerves through a surgical procedure. 1/3 of the biopsy specimens were placed in alcohol-formaldehyde-acetic acid (AFA) and 2/3 in glutaraldehyde. The specimens were sent to the laboratory for microscopic examination.

## Results

In the light microscopic examination of the inferior alveolar nerves in the saline group, the loss of integrity of the myelin sheath observed in the majority of samples. But some nerve sections were surrounded by epineurium. Myelin was regular in cases where the integrity preserved (Figure 3). In addition to the fibrous connective tissue of the epineurium, the presence of blood vessels was also noticed in these sections. In the sections where the integrity was preserved, nerve fascicles containing myelinated and unmyelinated nerve fibers were detected in the lower part of the epineurium. In the majority of the samples, the myelin sheath lamellae were irregular and distinct from each other, with significant degeneration in axons and myelin sheaths. In the samples where the myelin sheath integrity preserved, significant regeneration areas were observed in myelinated nerve fibers. Vacuole formation was detected in two samples. In addition,

there were degenerated nerve cells and macrophages with phagocytosed myelin sheaths in the degenerated areas. There was a significant decrease in the axon diameters and myelin sheath thicknesses in all sections, including the samples where the myelin sheath integrity preserved.

In the light microscopic examination of the inferior alveolar nerve samples in the dexamethasone group (Figure 4), the nerves were generally surrounded by epineurium. Macrophages were observed in the degeneration regions. In most of the samples where the integrity preserved, the myelin sheaths were significantly and severely irregular. Some of the samples demonstrated a regeneration in myelinated nerve fibers. Vacuole formation was observed in 6 of the samples. As in the saline group, axon diameters and myelin sheath thicknesses were decreased.

In the light microscopic examination of the inferior alveolar nerves in the vitamin B complex group (Figure 5), some nerve sections were surrounded by epineurium. But the majority of the samples were with integrity loss of the myelin sheath similar to the dexamethasone group. Even in cases where integrity was preserved, myelin sheaths were not regular. In the majority of samples, myelin sheath lamellae were disorganized and separated from each other, with significant degeneration in axons and myelin sheaths. There were regeneration areas in cases where the myelin sheath integrity was preserved. Vacuole formation was detected in 9 samples. In addition, degenerated nerve cells and macrophages with phagocytosed myelin sheaths were detected in the degenerated areas. There was a significant decrease in the axon diameters and myelin sheath thicknesses in all sections, including the samples where the myelin sheath integrity preserved.

In the light microscopic examination of the inferior alveolar nerves in the NGF group, the majority of nerve sections were surrounded by epineurium, and myelin sheath integrity was almost intact. The irregularity of myelin sheaths was also significantly lower than the other groups (Figure 6). While there was no significant degeneration in the axons and myelin sheaths in the samples, the myelin sheath lamellae were observed to be irregular and separated from each other, often seen in marked areas of regeneration. Vacuole formation was detected in 11 samples. Axon diameters and myelin sheath thicknesses were close to normal in all sections.

Statistical analysis was performed with SPSS version 17.0. The normal distribution of the variables was examined with the use of histogram graphs and the Kolmogorov-Smirnov test. Mean, standard deviation and median values were used in the descriptive analysis. Pearson Chi-Square and Fisher's Exact Tests were compared with 2x2 contingency tests. Kruskal Wallis Test was used to evaluate the nonparametric variables. Post hoc analyses were performed using the Mann Whitney U Test. P-values below 0.05 were evaluated as statistically significant results.

There was a significant correlation between the myelin sheath integrity rate ( $p: 0,013$ ) and the myelin sheath irregularity among the groups ( $p: 0,005$ ). The post hoc analysis was used to determine the significance of this group (Table 1). There was no statistically significant correlation between the formation of vacuoles (Table 2) ( $p > 0,050$ ).

## Discussion

In this study, crush type damages were created experimentally in the inferior alveolar nerve of rats. The aim of this study was to determine and compare the effects of local administration of dexamethasone, B vitamin complex, and nerve growth factor. Although there are clinical and animal studies that measure the regeneration capacity of the peripheral nerves, there are few studies on the inferior alveolar nerve. There are also few studies on the importance of local application. When inferior alveolar nerve damage occurs, the clinical approach is mostly on systemic applications.

The most commonly used drugs in peripheral nerve injuries are steroids. Steroids demonstrate an anti-inflammatory action by inhibiting the phospholipase A2, suppressing the macrophage inhibition factor, stabilizing the lysosomal and other membranes, granulocytes (Graham et al. 1973; Chuenkongkaew and Chirapapaisan 2002; Yates et al. 2004; Romundstad et al. 2004; Stubhaug et al. 2007; Suslu et al. 2013). Becker et al. reported that local steroid applications reduce the number of fibroblasts and scar tissue formations in peripheral nerve injuries (Becker et al. 1987). In addition, in the same study, they reported that steroids prevented the neuroma formation with these effects and stimulated myelin sheath production through the activation of Schwann cells. It was stated that they contribute to the regeneration by increasing axonal sprouting. Suslu et al. compared the systemic and local applications of dexamethasone on the sciatic nerve, they made crush-type damages to the nerve for 30 seconds and emphasized that the local application in the nerve regeneration was more successful than the systemic application (Suslu et al. 2013). In this study, as in reported studies, dexamethasone was found to have a positive effect on nerve regeneration when compared with the saline group in the light microscopic examination.

Clinically, there are many studies suggesting that the vitamin B complex reduces the degeneration of the nervous system. These vitamins, especially vitamin B12, play an important role in various biological events related to the condition of normal nerve functions (Jolivalt et al. 2009; Hobbenaghi et al. 2013). Besalti et al. reported positive results for motor functions related to the nerve regeneration in their study on rats' sciatic nerve (Besalti et al. 2007). Okada and Tanaka reported that the application of local or systemic high-dose vitamin B12 has a positive effect on the treatment of peripheral nerve damages in rats' sciatic nerve (Okada et al. 2010). In this study, a combination of B1, B6, and B12 vitamins was applied on the injured inferior alveolar nerve of rats. This application was found to support the regeneration process positively.

NGF is the first nerve growth factor to have been discovered and it is one of the most important neurotrophic factors in the study of nerve injuries (TERENGI 1999). NGF, which is normally at a very low concentration in the adult nerve, increases rapidly in Schwann cells after experimental nerve damage. This increase guides immigration of activated macrophages and interleukin-1 (Frostick et al. 1998). Wang et al. reported that neurotrophic factors have a significant effect on nerve regeneration after peripheral nerve injuries and are the most effective treatment agent with significant results obtained in studies (Wang et al. 2009). Fumagalli et al. reported that NGF promotes the regeneration of neurons and

axons and plays a key role in the repair of damaged nerves (Fumagalli et al. 2008). In this study, the effect of NGF on the regeneration of the inferior alveolar nerve was significant when compared to the other groups.

It can be concluded that when crush type nerve damage occurs in oral region, local administration of drugs instead of systemic administration will have more clinically effective results. Given that NGF produces more successful results than steroid and vitamin B complexes, which are preferred more in the clinical practice, the necessity of integrating growth factors into clinical procedures comes to the fore.

## Declarations

**Funding:** This work was supported by the Research Fund of Istanbul University. Project No. 24648

**Conflicts of Interest:** The authors declare that they have no conflict of interest.

**Ethics Approval:** All animal experiments in this study were conducted according to the Guidelines for Istanbul University Laboratory Animals Local Ethics Committee.

**Consent to participate:** Not applicable

**Consent for publication:** Not applicable

**Availability of data and material (data transparency):** The data that support the findings of this study are available on request from the corresponding author.

**Code availability (software application or custom code):** Not applicable

**Authors' contributions:** The authors declare that all data were generated in-house and that no paper mill was used. AYG, GU and GA designed the study. AYG and GA ran the experiments. GU made pathological examinations. AYG analysed the data. AYG, GU and GA wrote the manuscript.

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## Tables

Table 1: Comparison of the rate of myelin sheath integrity and myelin sheath irregularity between groups

	Myelin Sheath Integrity Distortion Ratio (%)			Myelin Sheath Disorder Ratio (%)		
	Average	s.d.	Median	Average	s.d.	Median
Saline	65,83	±48,70	100,00	68,75	±46,42	100,00
Dexamethasone	56,25	±49,78	90,00	83,33	±30,03	100,00
Vitamin B complex	42,08	±42,29	40,00	70,83	±27,87	77,50
NGF	4,17	±9,96	0,00	28,33	±23,96	25,00
p <sup>1</sup>	<b>0,013</b>			<b>0,005</b>		

**Table 2: Comparison of vacuol formation between groups**

	Vacuole Formation				p <sup>1</sup>
	Yes		No		
	n	%	n	%	
Saline	2	(50,00)	2	(50,00)	0,093
Dexamethasone	2	(25,00)	6	(75,00)	
Vitamin B Complex	0	(0,00)	9	(100,00)	
NGF	1	(8,33)	11	(91,67)	

## Figures

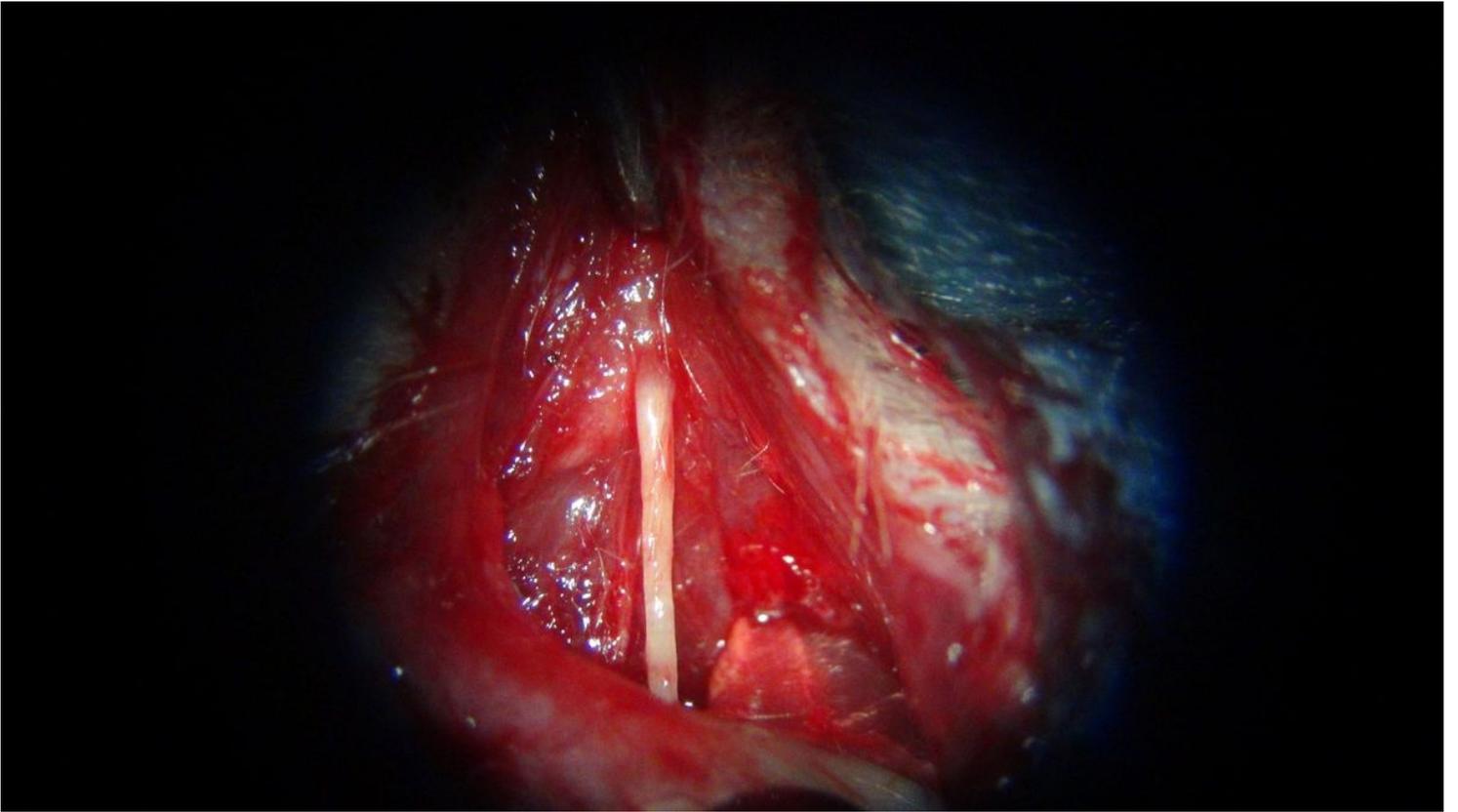
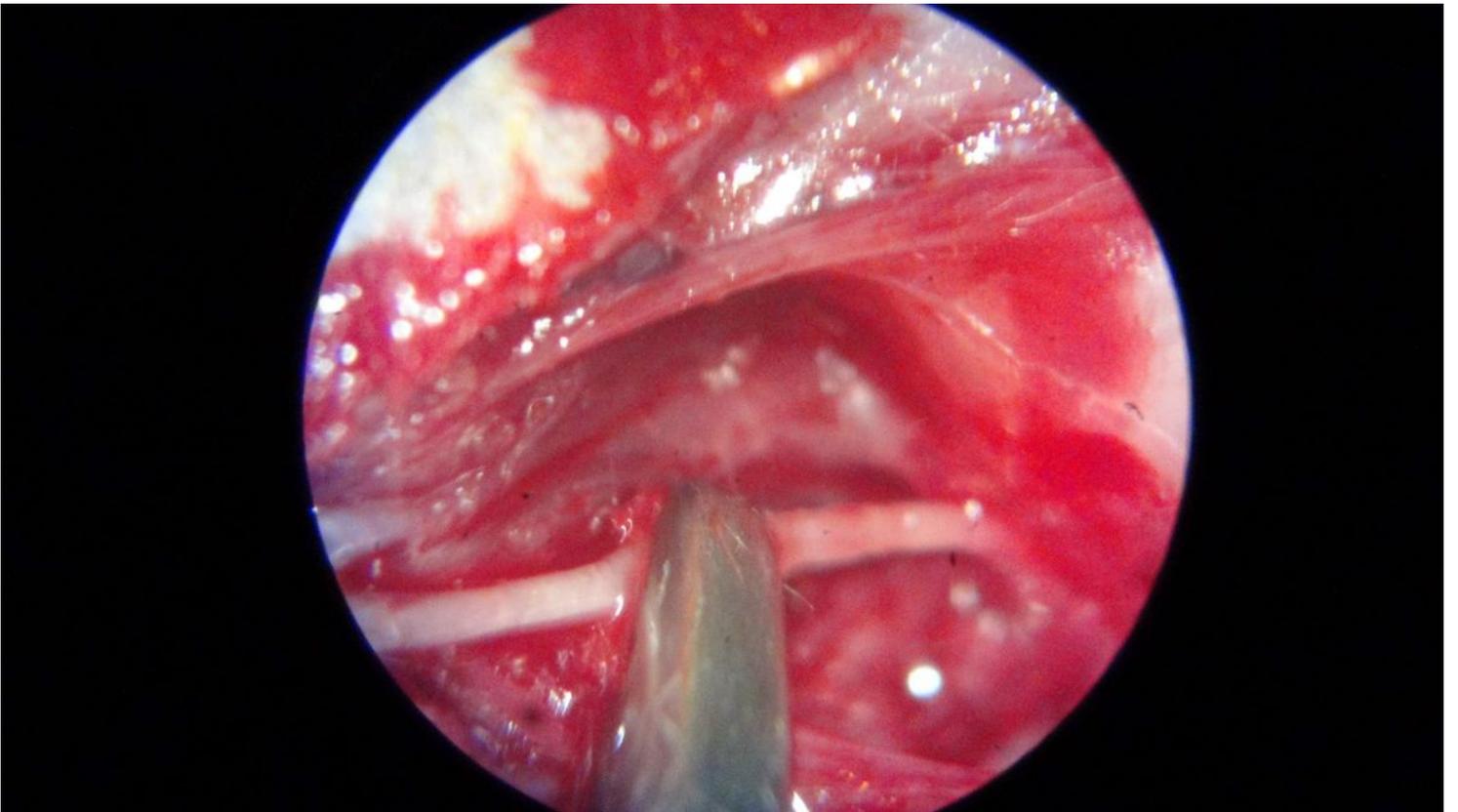


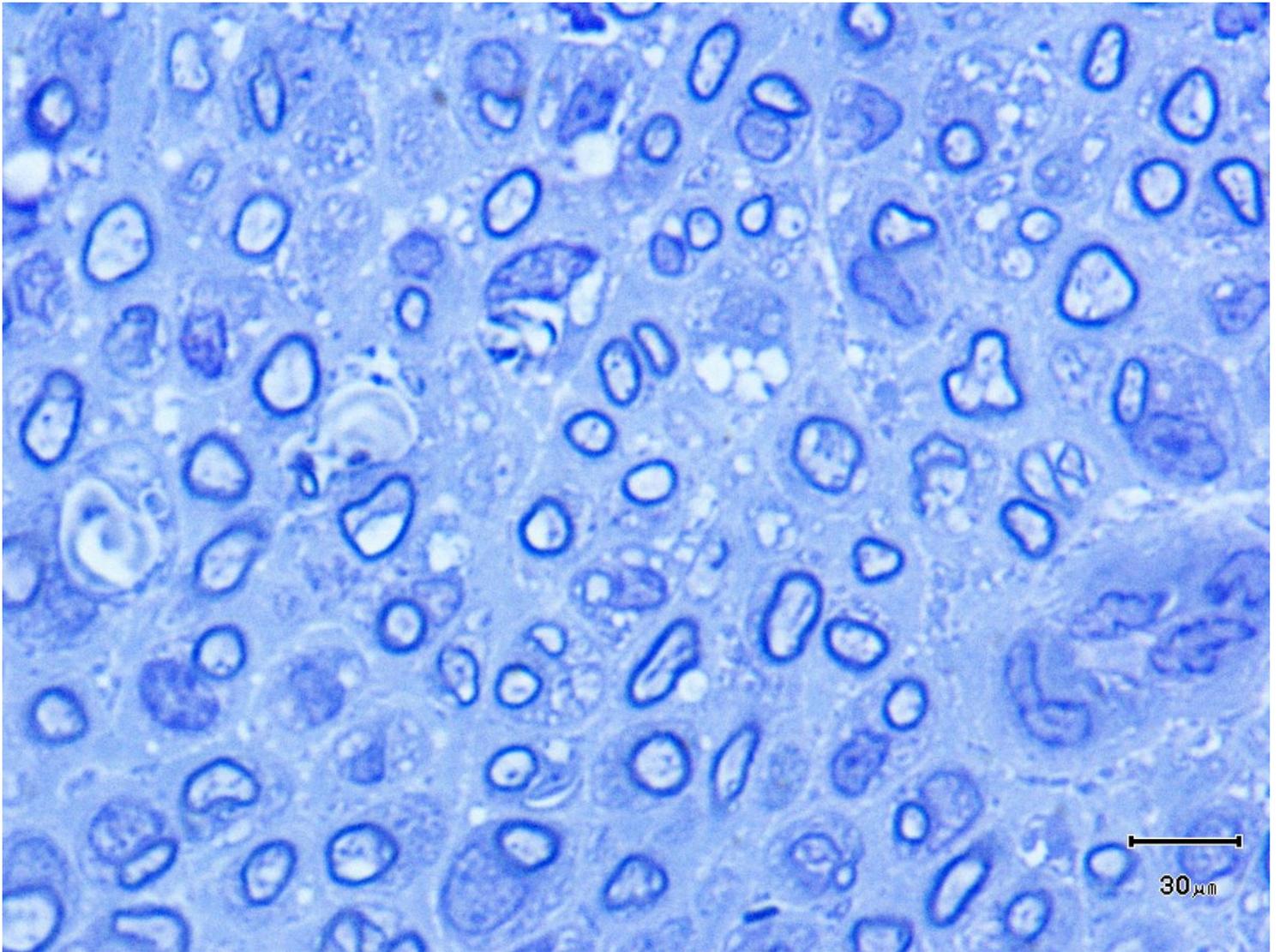
Figure 1

Image of exposed inferior alveolar nerve under surgical microscope



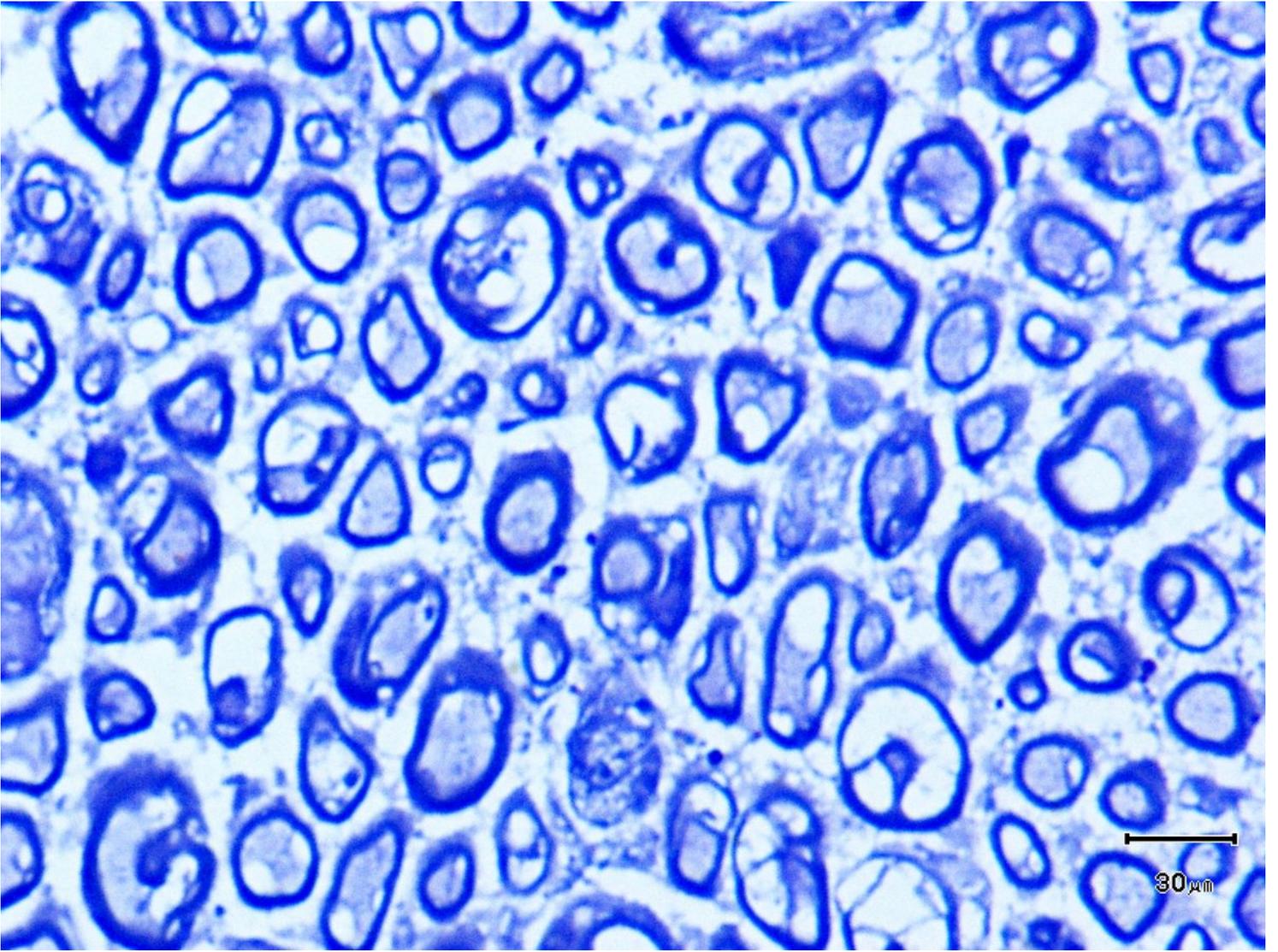
**Figure 2**

Image of surgical microscope during crush-type injury



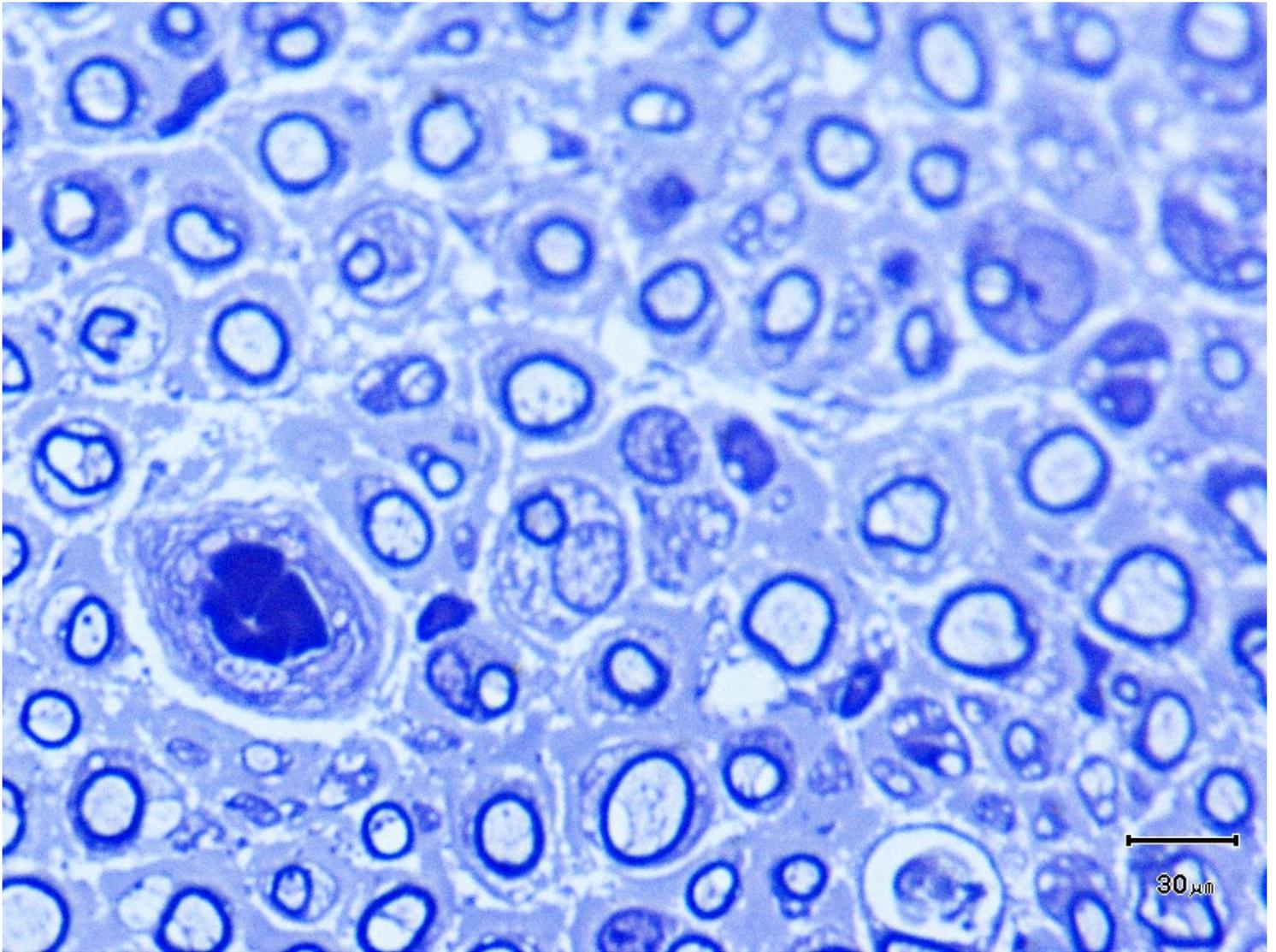
**Figure 3**

Section of myelin sheath with decreasing number of cells in saline group



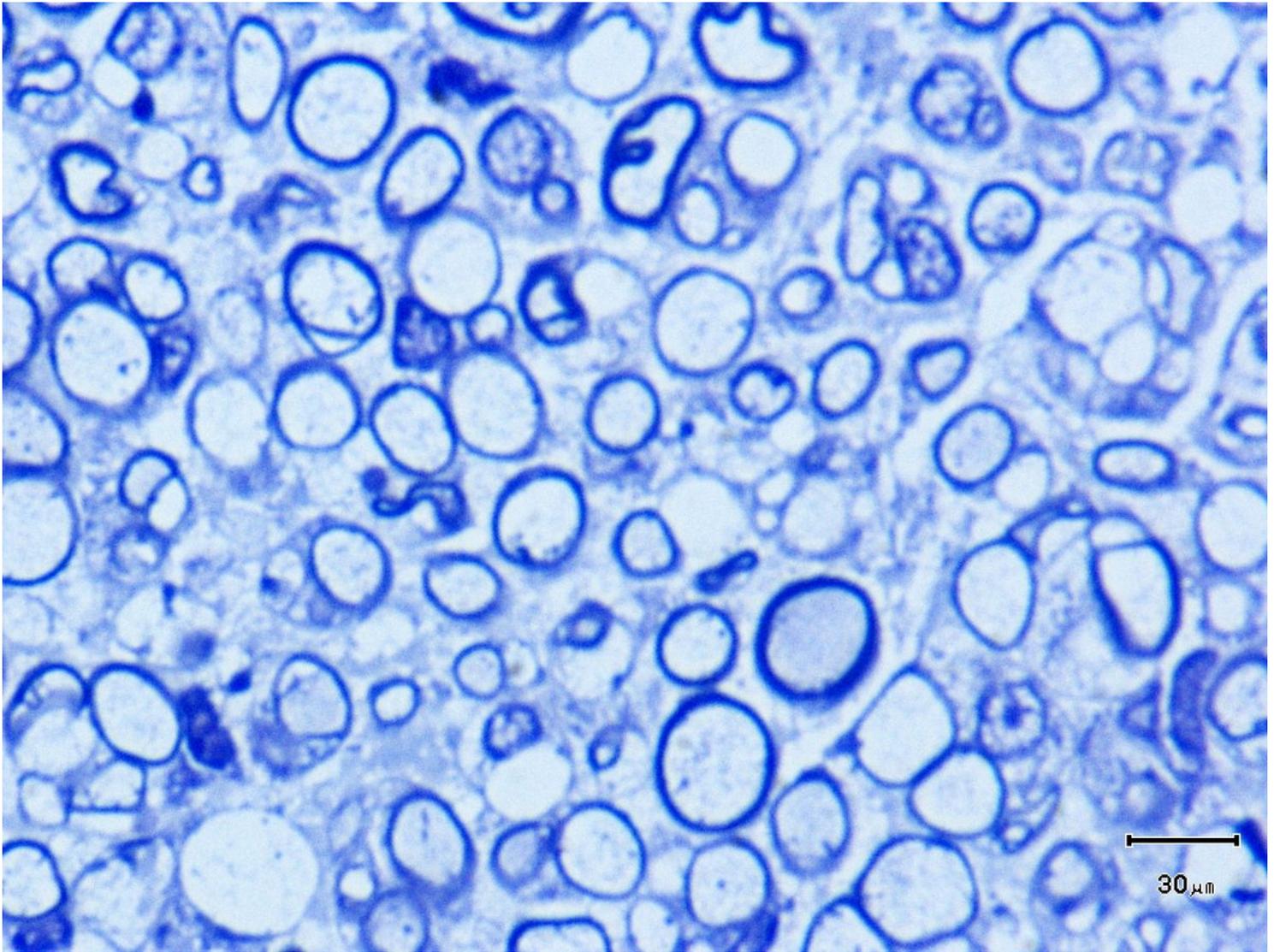
**Figure 4**

In the dexamethasone group, the myelin integrity began to deteriorate, myelin irregularities, the vacuoles



**Figure 5**

Damaged myelin sheath integrity and myelination irregularities with the degenerated areas in the B vitamin complex group



**Figure 6**

In the NGF group, the preserved myelin sheath integrity with no myelin irregularities