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The Effect of Hemostatic Agents and Tissue Adhesive on Injured Peripheral Nerve Healing in Rats – Part I. Electrophysiological Study*

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article; G – other

Abstract

Background. In the practice of maxillofacial surgery, bleeding and nerve injury have common problems. In the control of bleeding, hemostatic agents and tissue adhesives have been frequently used. The effect of these hemostatic agents and tissue adhesives on the injured neural tissues has not been known.

Objectives. In this study, we aimed to investigate the effects of hemostatic agents and tissue adhesive on injured nerve tissues.

Material and Methods. Forty-two rats randomly divided into seven groups: Control, Oxidized Regenerated Cellulose (ORC), Gelatine Sponge (GS), Bovine Collagen (BC), Ankaferd BloodStopper (ABS), Glutaraldehyde Surgical Adhesive (BioGlue®) and *N*-butyl-2 cyanoacrylate (Glubran®2). The left sciatic nerves were crushed and surrounded by hemostatic agents and tissue adhesives. At the end of 12 weeks, the surgical site was reopened and electrophysiological recordings were performed.

Results. In the ORC, GS, and BC groups, the compound action potential (CAP) values were lower compared to the control group ($p < 0.05$). Although the values of CAP in the ABS group were higher than in the control group while CAP values in the BioGlue and Glubran®2 groups were lower than the control group, there was no statistical significance between the experimental and control groups ($p > 0.05$). In the ORC, BC, GS, and Glubran®2 groups, the nerve conduction velocities (NCV) values were lower than in the control group ($p < 0.05$). In the ABS and BioGlue groups, NCV values were lower compared to the control group but no significant differences were found ($p > 0.05$).

Conclusions. The present study provides evidence that ABS is the most suitable hemostatic agent due to its favorable effect on the healing of injured neural tissues. BioGlue is also a suitable surgical agent with no adverse effects (*Adv Clin Exp Med* 2015, 24, 1, 23–29).

Key words: injured nerve tissues, hemostatic agents, tissue adhesives.

In the practice of maxillofacial surgery, many procedures, such as cyst or tumor excision, orthognathic surgery, implantology and impacted tooth removal can lead to injury of the peripheral nerve tissues [1–3]. Incidences of nerve damage after the removal of mandibular third molar teeth ranged from 0.4% to 8.4% [2]. A significant portion of patients with an injured peripheral nerve

develop sensory disturbances, such as persistent paresthesia, dysesthesia and pain. Although many classifications have been proposed in the past, the classification proposed by Seddon is the one currently in use. Seddon uses 3 classifications for peripheral nerve injury: neurapraxia, axonotmesis and neurotmesis [4]. The amount of nerve damage could be related to the surgical technique or

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the properties of the surgical medicaments inserted [5, 6].

In surgical procedures, hemostatic agents are frequently used to prevent intraoperative and postoperative bleeding, especially after the excision of a large pathologic lesion. During orthognathic or trauma surgery, peripheral nerves can be visualized and hemostatic agents are sometimes placed in close proximity to peripheral nerves [6, 7].

Oxidized regenerated cellulose (ORC) is a chemically altered form of cellulose. It is normally used to control oozing from broad surfaces, but it can also be applied directly to brain surfaces. Its physical properties are loosely knit and allow placement in certain areas where it will rapidly conform to the recipient's surface. Gelatin sponge (GS) is made from animal skin, gelatin whipped and baked into sponge form. GS is widely used to fill the cavity in a bloody field [7]. Bovine collagen (BC) is derived from purified bovine corium. The classical form (flour form) is water insoluble, partially composed of acid salt from collagen that is processed into microcrystals. It is dry, fluffy, white, and self-adherent [7].

Ankaferd BloodStopper® (ABS) is a folkloric herbal mixture that has been used for hemostasis. It is a standardized plant mixture. The basic mechanism of ABS is the formation of an encapsulated protein network, which provides focal points on which vital erythrocytes can aggregate. The ability of ABS to induce formation of a protein network not only makes it an effective hemostatic agent, but also confers anti-infective, antineoplastic, and healing modulator properties to the extract. ABS represents an alternative treatment modality for many kinds of bleeding that are resistant to conventional methods [8].

The use of surgical adhesives and sealants to strengthen suture lines, improve hemostasis and serve as adjuncts in various surgical operations has increased since they first became commercially available. On the basis of their composition, they can be classified as natural, synthetic or combined preparations and further divided into fibrin, collagen, cyanoacrylate, polyethylene glycol, albumin-glutaraldehyde or gelatin-resorcinol-formaldehyde products [9].

BioGlue® is a surgical adhesive composed of 10% glutaraldehyde and 45% purified bovine serum albumin, which is commonly used in cardiovascular operations. Glubran®2 tissue skin adhesive is a sterile, ready-to-use cyanoacrylate adhesive for the skin. These products have considerable hemostatic and adhesive properties and act as an antiseptic barrier to infectious organisms in surgical operations [9, 10].

Many studies have been done on the effectiveness of different local hemostatic agents on the

peripheral neural tissues. However, to our knowledge, the effect of these hemostatic agents and tissue adhesives on the injured neural tissues has not been studied. Therefore, we investigated the effects of seven medicaments on injured nerve tissues.

Material and Methods

Animals

Forty-two albino female Wistar rats age 6 weeks weighing 200 to 250 g were used in this study. The animals were kept in standardized temperature and light conditions and randomly divided into 7 groups: Control, Oxidized Regenerated Cellulose (ORC) (Surgicel®; Ethicon, Neuchatel, Switzerland), Gelatine Sponge (GS) (Gelatamp; Roeko, Langenau, Germany), Bovine Collagen (BC) (Lyostypt®; Braun, Melsungen, Germany), Ankaferd BloodStopper® (ABS) (Ankaferd Health Products Ltd., Istanbul, Turkey), Glutaraldehyde Surgical Adhesive (BioGlue®; Cryolife Inc., Kennesaw, GA, USA), and *N*-butyl-2 cyanoacrylate (Glubran®2; GEM S.r.l., Viareggio, Italy). The rats were housed 2 per cage in a room, and they were fed specific rat chow and water *ad libitum*.

Surgical Procedures

The animals were anesthetized with an intramuscular administration of a mixture of ketamine hydrochloride (90 mg/kg; Ketalar®, Parke-Davies, Barcelona, Spain) and xylazine (10 mg/kg; Rompun; Bayer Vital, Leverkusen, Germany). The left

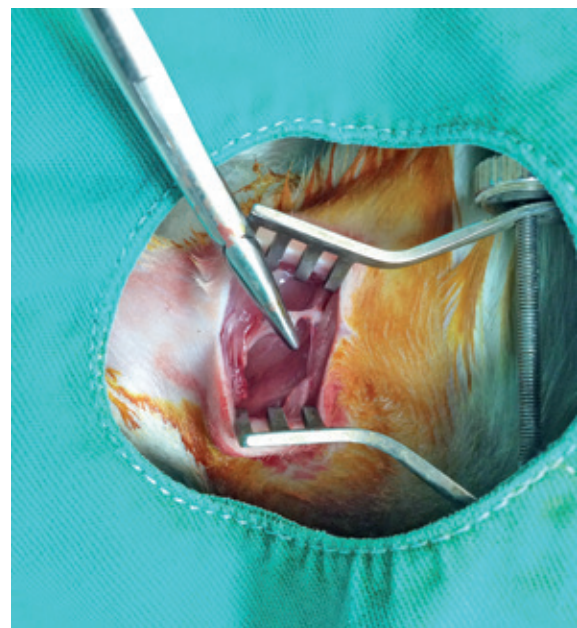


Fig. 1. The hemostat was applied for 1 min using approximately 50 N holding force

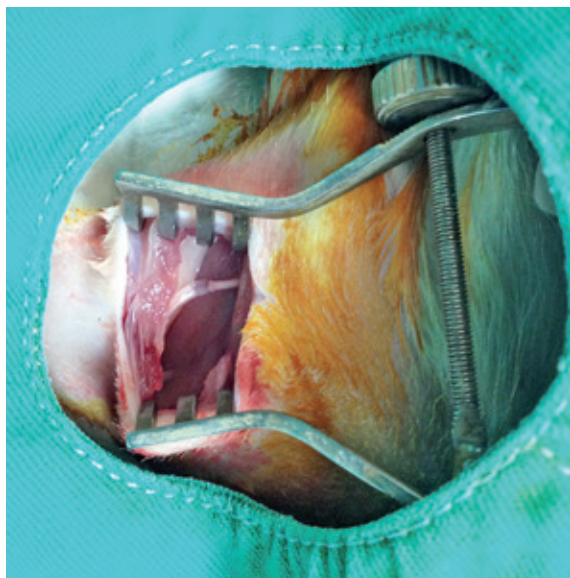


Fig. 2. The appearance of the injured peripheral nerve tissue

thigh, hip, and flank were shaved and swabbed with antiseptic solution (20% iodine solution).

Following one longitudinal straight cutaneous incision in the back thigh, blunt dissection was performed between the gluteus maximus and quadriceps muscles. The nerve was dissected until the tibial, peroneal, and sural branches were exposed.

We exposed approximately 2.5 cm of the sciatic nerve. The nerve was isolated from the surrounding tissues without injuring the epineurium. Both legs were fixed very well. The left sciatic nerves were crushed by using a special hemostat (Bahadır, Samsun, Turkey). The hemostat was applied for 1 min using approximately 50 N holding force (Fig. 1, 2). Then, the injured nerve was surrounded by ORC, GS, and BC, which was left in the area. In the ABS group, 2 mL of ABS was used with a sterile sponge and left in the injured nerve area for 3 min. In the BioGlue and Glubran®2 groups, tissue adhesives were dropped on the injured nerve and allowed 3 min to harden. After nerve injury in the control group, the surgical site was closed without any further procedures. After the surgical operation, the muscle and skin were closed with 4/0 vicryl and 4/0 silk suture. At the end of 12 weeks, the surgical site was re-opened and the electrophysiological recordings were performed in all groups. The study was approved by the institutional animal care and ethics committee of Ondokuz Mayıs University.

Electrophysiology

The following tests were performed according to a study by Alkan et al. [6]. The nerve potential recordings were carried out using an

electrophysiological data acquisition system (PowerLab 4/SP; AD Instruments, Castle Hill, Australia). Bipolar hook electrodes were used to both stimulate the left sciatic nerve and to record the nerve potentials. A hook electrode was placed proximate to the sciatic notch for the first stimulating point. The recording electrode was placed distal to the sciatic nerve. Primarily, the distance was 10 mm between the stimulating point and the recording electrodes and the latency of CAPs were measured through our scope program. After stimulating at the first point (10 mm from the proximal), the stimulating electrode was moved to the second stimulating point (10 mm from the first point). The recording electrode was not moved during the stimulating process. Supramaximal stimuli (10 V) of 0.2 ms in duration were delivered to the sciatic nerve from a stimulator on a PowerLab 4/SP data acquisition system.

The responses were amplified (BIO Amp, AD Instruments) and stored on a computer. Scope software (AD Instruments) was used for data capture and analysis.

The latencies were measured from the stimulus artifact to the onset of the negative wave deflection. Nerve conduction velocities (NCV) were calculated by taking the distance between stimulating electrodes and dividing it by the average latency difference between the onsets of the compound action potential (CAP). The amplitude of the CAP was measured from the base line to peak. To determine the latency, electrical stimulation was repeated 10 times and averaged per rat. For all groups, the NCV and CAP values were recorded at 0, 15, 30, 60, and 120 min. To determine the latency, electrical stimulation was repeated 10 times and averaged per rat. The animals were sacrificed after the records were obtained.

Statistical comparisons were made by using SPSS 16.0 (SPSS, Chicago, IL, USA) software. Data analysis was performed using one-way analysis of variance (ANOVA). The Tukey and Tamhane tests were used for comparisons, with values of $P < 0.05$ determined as statistically significant.

Results

The NCV and CAP values were evaluated. There was no difference between 0 and the 120th min. Therefore, statistical evaluation was performed according to the 0 min. The groups' mean NCV and CAP values are shown in Fig. 3 and 4. There are no overlap and artifact problems in the present study (Fig. 5, 6)

In the ORC, GS, and BC groups, CAP values were lower compared to the control group

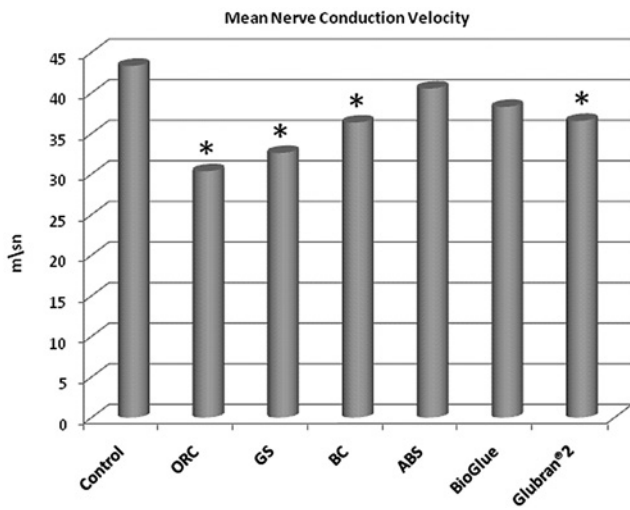


Fig. 3. The effects of different hemostatic agents on mean nerve conduction velocity, * statistically significant difference ($p < 0.05$)

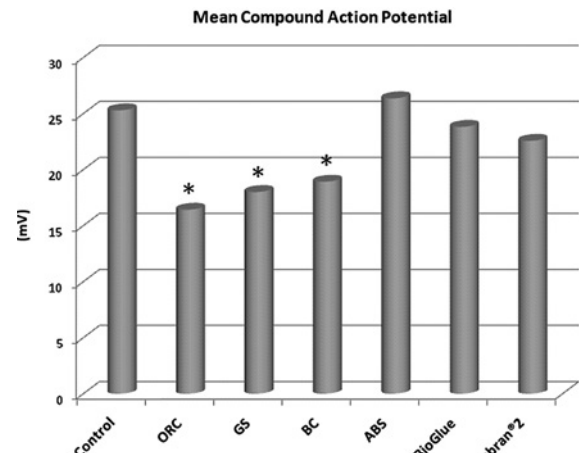


Fig. 4. The effects of different hemostatic agents on amplitude of the compound action potentials, * statistically significant difference ($p < 0.05$)

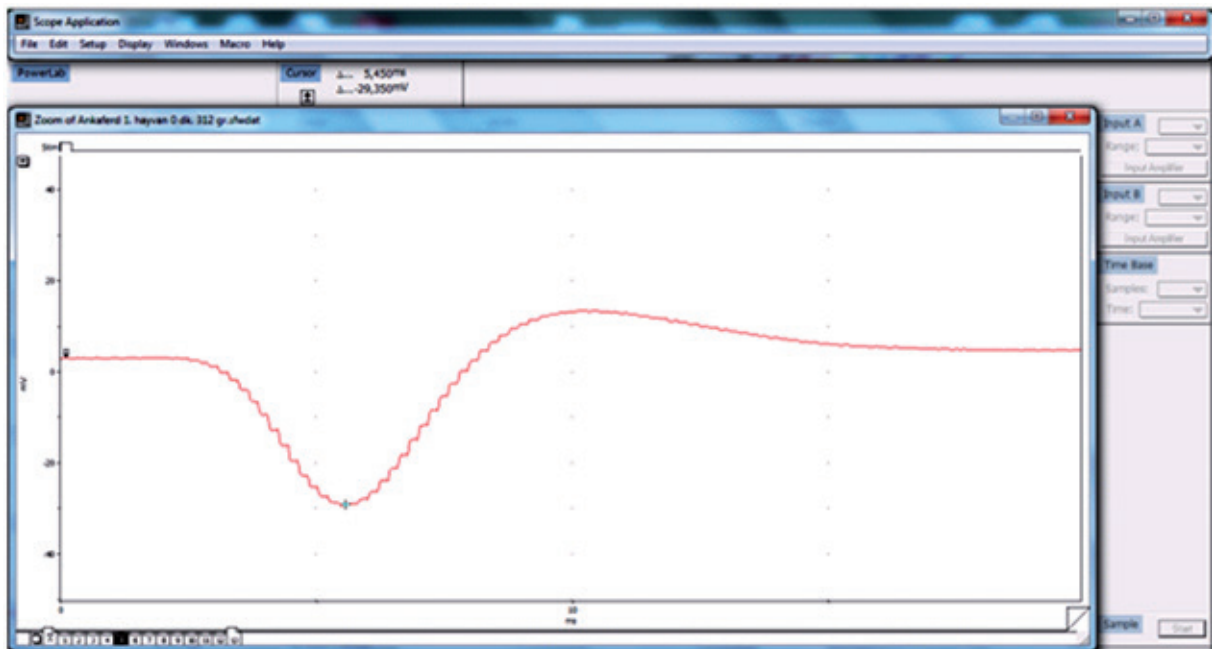


Fig. 5. One can see the value of amplitude (29.350 mV) and latency (5.450 ms) at the top of the screen

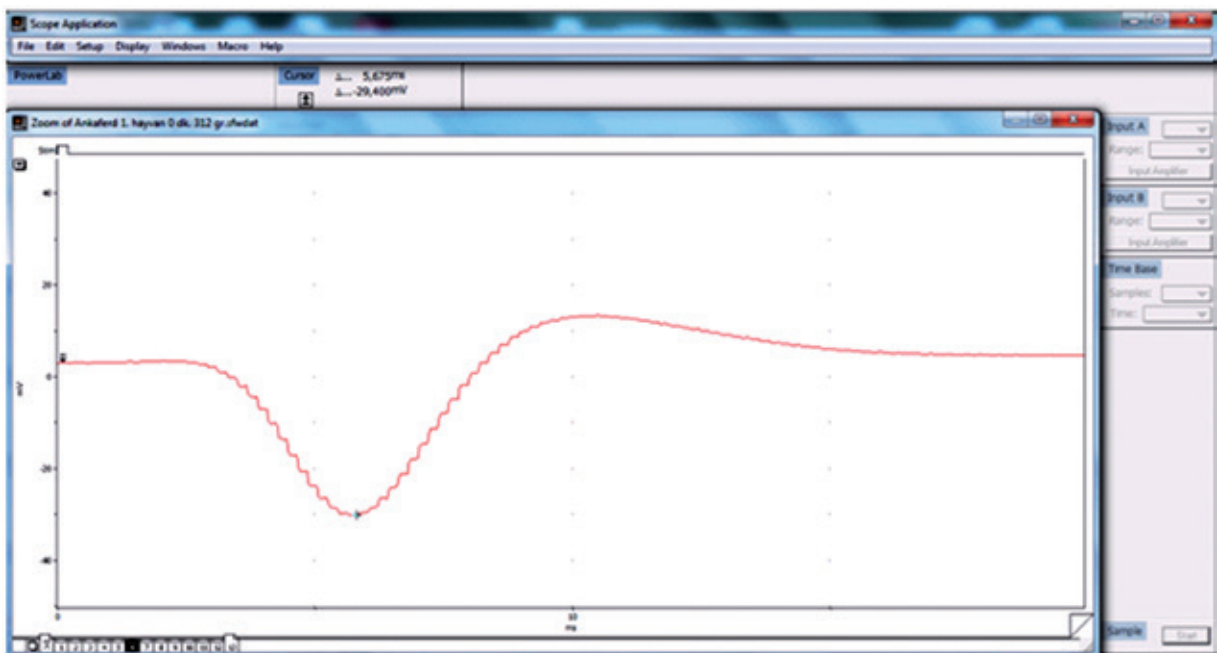


Fig. 6. One can see the value of amplitude (29.400 mV) and latency (5.674 ms) at the top of the screen

($p < 0.05$). Although the values of CAP in the ABS group were higher than in the control group while CAP values in the BioGlue and Glubran[®]2 groups were lower than in the control group, there was no statistical significance between the experimental and control groups ($p > 0.05$).

In the ORC, BC, GS, and Glubran[®]2 groups, NCV values were lower than the control group ($p < 0.05$). In the ABS and BioGlue groups, NCV values were lower compared to the control group but no significant differences were found ($p > 0.05$).

Discussion

Peripheral nerve injuries have always been a critical clinical problem in surgical procedures and remain a major cause of morbidity. In the maxillofacial region, injury to the peripheral nerves is a major complication and can result from a variety of clinical circumstances. The most common circumstances occur in dentoalveolar, cyst, tumor and orthognathic surgery. Possible mechanisms of nerve injury in patients who sustain sensory deficits postoperatively to intact nerve bundles include compression injury or crush injury [7, 11, 12].

The control of bleeding is an important step in oral and maxillofacial surgical procedures since severe hemorrhages may occur during such operations. Therefore homeostatic agents are used to aid hemostasis in both soft and hard tissues, and these are often placed in direct contact with neural tissues due to the vessels being situated in the associated neurovascular bundle [5].

Several studies have examined the response of neural tissues to hemostatic agents. Considering the previously reported studies, the short-term effects of the surgical medicaments on intact nerve tissues were examined in all studies [5–7]. We examined the effects of six different surgical medicaments on injured nerve tissues, since nerve damage is a common complication in maxillofacial surgical procedures. Additionally, electrophysiological recordings were made at the end of 12 weeks, since it is known that nerve healing is generally completed in three months [13].

There are some studies in the literature about the effects of ORC on peripheral neural tissues. Several authors reported paraplegia and neuropathy after the use of ORC, which is highly acidic. However, they stated that this effect might result from the use of excess ORC. The data obtained from the studies also suggested that the effects of ORC on neural function are only temporary [6, 7, 14]. To our knowledge, there are only a few studies about the effect of ORC on nerve function in the maxillofacial region [6, 14, 15]. Loescher and

Robinson reported a temporary effect of ORC on nerve function [15]. Alkan et al. [6] reported an immediate effect of ORC on nerve function, 1 h after the surgery. This study stated that CAP and NCV values regained characteristics that were similar to those of controls after 4 weeks. However, the above-mentioned studies didn't check the features of nerve conduction after long term healing. In contrast to previous results, the CAP and NCV values dramatically decreased in the ORC group in the present study. The difference may be due to both the time points tested and the injured nerve tissues that were used in our study.

Alkan et al. [6] observed the delayed effects of GS on nerve function. They reported that CAP was significantly higher 4 weeks after application in the GS group. In the present study, NCV and CAP values significantly decreased in the GS group compared to the control group. The effects of hemostatic agents on peripheral nerve function could be considered time-sensitive. Therefore, the results of the present study differ from the previous studies in that BC has no reported adverse effects on peripheral nerve function and tissue healing. Both Alkan et al. [6] and Pampu et al. [14] stated that BC has a beneficial effect on peripheral nerve function. In contrary earlier reports, our findings demonstrated that BC resulted in a remarkable negative alteration in CAP values compared to the control group.

ABS has been effectively used in oral surgery and other surgical procedures. It has been reported that ABS provides tissue oxygenation and physiological hemostasis without affecting any individual clotting factor. Additionally, it decreases inflammation and necrosis [8, 9]. This unique mechanism of action provides an advantage to ABS over other hemostatic agents [8, 9]. Currently, there is only one study about the effect of ABS on peripheral nerve function [14]. Pampu et al. [14] state that ABS demonstrated acceptable features compared to the other tested hemostatic agents on peripheral nerve functions. In line with the earlier report, the present study revealed that ABS has a positive effect on nerve healing and causes a mild increase in CAP values compared to the ORC, GS, and BC groups. In spite of that, CAP values were higher in the ABS group than in the control group. However, it is important to note that there was no statistical difference between the ABS group and the control group concerning the CAP values. Additionally, the NCV values of the ABS group were proximate to the control group. Therefore, our findings suggest that ABS has a beneficial effect on nerve regeneration, and it is a safe and reliable hemostatic agent for use in maxillofacial surgery.

Little data about nerve function problems after the use of tissue adhesives in the maxillofacial surgical area is available in the literature. Some studies have reported that BioGlue can cause local toxicity and extensive scarring [10, 16]. Lemaire et al. [10] reported that BioGlue causes acute nerve injury and myocardial necrosis after 3 to 60 min of nerve exposure. In the present study, BioGlue did not alter the injured peripheral nerve function; it exhibited values similar to the control group at the 12-week period ($p > 0.05$). In addition, no significant differences in CAP values were observed between the Glubran[®]2 group and the control group in this period ($p > 0.05$). The results of this study revealed no undesirable effects of tissue adhesives on nerve function compared to previous studies. This may be due to the time points tested. Unfortunately, we are not able to compare the results of the Glubran[®]2 group to previous studies since there is no study that has been done on nerve function using this substance.

To the best of our knowledge for the first time, the results of the present study show that ABS, BioGlue, and Glubran[®]2 have healing effects on peripheral nerve injury concerning CAP activity. ABS and BioGlue also have a healing effect on peripheral nerve injury concerning NCV

activity. Comparing the effects of surgical medicaments on nerve healing using injured nerve tissue has not been previously reported. In the present study, regenerating effects were also observed in the later period (12 weeks). Our study confirmed that ABS had a positive effect on nerve healing. BioGlue played a favorable role in NCV values. The mean NCV values of the Glubran[®]2 and control groups showed similar values but when the groups were compared, significant differences were found. ORC, GS, and BC had a negative effect on nerve healing. Most nerve injury is due to the mechanical compression of neural structures because of the blind application of local agents into closed bone compartments or the natural swelling of those products. The possible adverse effects of surgical medicaments on nerve function should be considered.

In conclusion, this study provides evidence that ABS is the most suitable hemostatic agent due to its favorable effects on the healing of injured neural tissues. BioGlue could be considered an acceptable surgical medicament as it demonstrates no adverse effects on the healing of injured neural tissues. Further histological and histomorphometric research should be conducted to explain the effects of these agents on injured nerve tissues.

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