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Effects of Platelet-Rich Fibrin Membrane on Sciatic Nerve Regeneration

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Abstract: Alternative treatment approaches to improve the regeneration capacity of damaged peripheral nerves are currently under investigation. The objective of the present study was to evaluate the effects of platelet-rich fibrin (PRF) membrane after sciatic nerve crush injury in rabbits by histomorphometric and electromyographic analysis. The left sciatic nerves of 20 male Vienna rabbits were clamped for 30 seconds to induce crush injuries. Animals were randomly divided into 2 groups: PRF and control. For each animal in the PRF group, a PRF membrane was wrapped around the injured part of the sciatic nerve to form a tube. No additional treatment was performed in the control group. After a 12-week healing period, tissue samples from the injured nerve region were harvested and the g-ratio of axons, axon density, and impulse transmission changes were evaluated. Analysis revealed that axon density differences were not statistically significant between groups ($P = 0.139$). The rate of nerve fibers with optimum g-ratio was significantly lower in the PRF group than in the control group ($P = 0.02$). Conduction velocity differences between groups were not statistically significant. Although PRF application has previously shown positive regeneration effects on maxillofacial tissues, local PRF membrane application in tube form did not show any histomorphometric or functional improvement in peripheral nerve crush injury recovery.

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Received June 27, 2017.
Accepted for publication October 6, 2017.

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This study was supported by the Baskent University Research Fund.
The authors report no conflicts of interest.

ISSN: 1049-2275
DOI: 10.1097/SCS.0000000000004256

Key Words: Nerve tubulization, peripheral nerve regeneration, platelet-rich fibrin, platelet-rich fibrin membrane

Damaged peripheral nerves commonly need surgical intervention to obtain satisfactory functional recovery.[1] Although there have been great improvements in microsurgery, peripheral nerve repair is still a challenge for surgeons. In short gaps, primary edge-to-edge repair is the most effective microsurgical technique, whereas autogenous nerve graft placement is accepted as the gold standard in long damage gaps.[2,3]

The tubulization technique includes the wrapping of the nerve repair site with tubular structures that may or may not contain substances that promote axon regeneration. Tubulization can act as a guide for bridging and provide a regenerative microenvironment for cellular proliferation. The tubulization technique has been identified as an effective alternative treatment option to autogenous nerve graft in recent years.[4]

The most frequent types of peripheral nerve damage in the field of oral and maxillofacial surgery are contusion and compression.[1] Possible causes of this nerve damage include maxillofacial trauma, orthognathic surgery, dentoalveolar surgery, surgical removal of the impacted lower third molar, implant placement, and injection of local anesthesia.[5–8] The development of alternative approaches is necessary to improve the recovery process from this nerve damage.

Platelet-rich fibrin (PRF) was first developed at the beginning of the 21st century by Choukron et al in France.[9] PRF can release various types of growth factors (GFs) such as platelet-derived growth factor (PDGF), transforming growth factor-β1 (TGF-β1), epidermal growth factor, and vascular endothelial growth factor (VEGF) via platelet activation for 28 days following application.[9] It is a natural autologous fibrin matrix that promotes chemotaxis, regeneration, and wound healing. PRF can be easily obtained as a membrane or in solution form (injectable PRF) and applied to a wound or damaged surface.[10]

The effect of platelet-rich plasma (PRP) on peripheral nerve regeneration was previously investigated in several studies in animal models.[11–14] Lichtenfels et al[13] reported that when PRP and PRF were applied to a region of damage with a silicon tube, they had positive tubulization effects on functional sciatic nerve recovery. However, Senses et al[15] reported that PRF membrane application decreased functional recovery in a transected sciatic nerve injury model. Given these incompatible findings, the effects of platelet concentrates on peripheral nerve regeneration are still controversial.

To the best of our knowledge, this study is the first to evaluate the isolated effects of PRF membrane on crushed peripheral nerve. The aim of the present study was to investigate the histological and physiological effects of isolated PRF membrane on the regeneration of sciatic nerve crush injury in a rabbit model.

MATERIAL AND METHODS

Animals and Surgical Procedure

This study was approved by the Institutional Review Board and Ethical Committee of Baskent University for experimental research on animals (Project No: D-DA 13/07) and supported by the Baskent

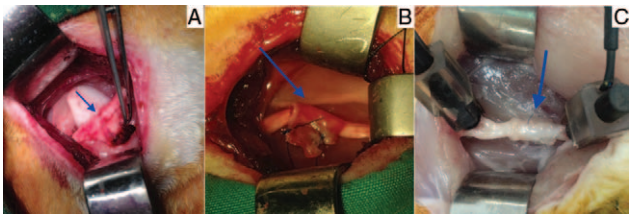

FIGURE 1. Surgical and electrophysiological methods. (A) The crush injury region on the left sciatic nerve. (B) Stabilized PRF membrane in tube form around the nerve injury region. (C) Placement of distal and proximal electrodes for evaluation of conduction velocity speed and amplitude deflection. PRF, platelet-rich fibrin.

University Research Fund. Twenty male 1-year-old Vienna rabbits were obtained from the animal experiment center of Baskent University. Animals were kept on a 12-hour day/night cycle and maintained ad libitum on water and standard laboratory aliment. Animals were anesthetized twice by intraperitoneal injection of ketamine (45 mg/kg) and xylazine (7 mg/kg) combination. The first anesthesia was performed for initial experiments and the second was for the 12-week postoperative evaluations. Preoperative enrofloxacin (10 mg/kg, intramuscular) and fentanyl (0.02 mg/kg, subcutaneous) were also given. For all animals, the skin on the lateral part of the left thigh was shaved and disinfected with povidone iodine. After making oblique skin incisions and blunt muscle dissections, the sciatic nerve was bluntly separated along the spatium intermusculare. Soft tissue around the sciatic nerve was also dissected for optimal electrophysiological measurements. The left sciatic nerve was crushed 2 cm distal to the sciatic notch. Crush injuries were performed with a hemostatic clamp for 30 seconds. The second level of the rack was utilized to maintain the nerve crush (Fig. 1A). To preserve animals' mobility, the right sciatic nerve was not operated on.

Animals were randomly divided into 2 groups of 10. Animals in the first group received local PRF treatment; this study group was designated the PRF group. No treatment was applied to animals in the second group, which was designated the control group. For the PRF group, 8-mL autologous blood samples were collected from the ear vein in a sterile tube without anticoagulant and immediately centrifuged at 2700 rpm for 12 minutes. To obtain PRF membranes, PRF gels were compressed in a PRF box (PRF Box Process, Nice, France) that was specially designed to shape PRF gels. The injured part of the sciatic nerve was wrapped with a PRF membrane, which was then sutured to surrounding muscles for stabilization (Fig. 1B). For both groups, surgical incisions were closed with 4.0 vicryl sutures in a layered fashion. A postoperative antibiotic (enrofloxacin, 10 mg/kg, intramuscular, twice daily) and analgesic (fentanyl, 0.02 mg/kg, subcutaneous, twice daily) were administered for 5 days to prevent infection and provide analgesia. Animals were returned to standard cages and laboratory conditions. At the end of the 12-week healing period, animals were anesthetized for a second time and access to the sciatic nerve was achieved with the same surgical procedure. After final electromyographic (EMG) measurements, nerve samples from the distal part of the injury were harvested and animals were sacrificed with an overdose of ketamine (150 mg/kg).

Histological and Histomorphometric Examination

Harvested tissues from both groups were fixed in a phosphate buffered solution (ph 7.3) containing 2.5% glutaraldehyde (Sigma-Aldrich Co, St. Louis, MO) for 2 hours at room temperature, postfixed in 1% osmium tetraoxide (Sigma-Aldrich Co), and dehydrated in a series of graded alcohols (50%, 60%, 70%, 80%, 90%, and 100% ethanol). After passing through propylene oxide (Sigma-Aldrich Co), specimens were embedded in Araldite CY 212 (Ciba-Geigy, Delhi, India), (2-dodecen-1-yl) succinic anhydride (Sigma-Aldrich Co), benzyldimethyl amine (Poly Sciences Inc, Philadelphia, PA), and dibutyl phthalate (Sigma-Aldrich Co). The semithin sections were stained with toluidine blue (Sigma-Aldrich Co) and examined with a photomicroscope (DM 500 Leica, Wetzlar, Germany). After the selection of appropriate specimens, thin sections were cut and stained with uranyl acetate (ProSciTech, Townsville, Australia) and lead citrate (Sigma-Aldrich Co). They were histologically examined with an electron microscope at 6000× magnification by 2 histologists who were blinded as to sample group (Leo 906 E Carl Zeiss, Göttingen, Germany).

Histomorphometric evaluation of the g-ratio for myelinated axons was made in the semithin sections at 1000× magnification. Figures of randomly selected fields of transverse sections were drawn. G-ratio values between 0.55 and 0.68 were considered to be in the optimal range.[16] The g-ratio was calculated by dividing the inner axonal diameter by the outer fiber diameter, and the results were stratified in ranges of 0 to 0.1, 0.1 to 0.2, 0.2 to 0.3, 0.3 to 0.4, 0.4 to 0.54, 0.55 to 0.68, 0.69 to 0.8, 0.8 to 0.9, and 0.9 to 1. Quantifications were performed using the Tantuna 76 software (Baskent University Department of Biophysics, Ankara, Turkey). This program has been specifically developed to calculate the g-ratio in peripheral nerve research. Axon number per unit area was evaluated in the semithin sections at 100× magnification using Image-J software (ImageJ 1.48v; National Institutes of Health, Bethesda, MD). The total number of axons in each section was counted and divided by the total area.

Electrophysiological Examination

EMG measurements were performed at 2 time points for both groups: before inflicting the crush injury and at the end of the 12-week healing period. Two platinum electrodes were placed tightly on the nerve approximately 1.5 cm apart from each other (Fig. 1C). Two consecutive rectangular waves of 6 V with 0.2-ms duration, 2-ms latency, and 20-ms interval were applied via a stimulator (STPT 05, May Research stimulator, Commat Ltd, Ankara, Turkey). The combined action potential was then recorded via a computerized physiological data acquisition system (BioPac, MP100, Commat Ltd). Nerve responses were recorded to determine the supramaximal parameters for the nerve stimulation process. Nerve conduction speed was calculated by dividing the distance (cm) by Δ time (ms), and expressed in m/s as V1, V2, V3, and V4. Four intervals on the trace were assessed (Fig. 2).

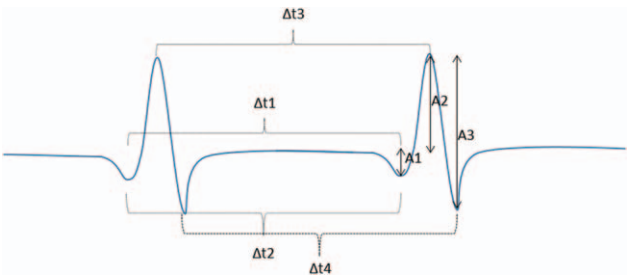

FIGURE 2. Investigated time intervals and amplitudes for electrophysiological results. The combined action potential after a stimulus (2 rectangular waves of 6 V, 2 ms latency, 0.2 ms duration, 20 ms interval) recorded at the rabbit sciatic nerve in vivo. Δ*t* indicates the time period at respective intervals. A1, A2, and A3 indicate the amplitude of the first negative deflection from baseline, first positive deflection from baseline, and the amplitude from the peak to the trough of the last deflection, respectively.

Statistical Analysis

Sample size was calculated for this study by paired-means power analysis to ensure at least 90% power with a significance level of 0.05. According to the power analysis results, 10 rabbits per group provided 92% power. The g-ratio results are expressed as numbers (n) and percentages (p). Categorical data were analyzed with Fisher exact test and the chi-squared test. Where expected frequencies were <5, the Monte Carlo simulation method was performed. The EMG and axon density results are expressed as mean ± SD. Independent-sample *t* test was used to compare axon density results between the PRF and control groups. One way ANOVA for repeated measures was used to analyze differences in preinjury EMG results between the control and PRF groups. *P* values <0.05 were considered to indicate statistical significance. Statistical analyses were carried out using PASW 18.0 software (SPSS, Chicago, IL).

RESULTS

Recovery from anesthesia and surgical interventions was uneventful for all rabbits. No postoperative complications (such as infection, bleeding, or allergic reaction) were noticed in the surgical area. However, due to decreased mobility, stage II pressure ulcers were observed at the left hocks of 3 rabbits.

Histological and Histomorphometric Results

Degenerative changes—such as demyelination in the large-diameter axons and separation between the axonal membrane and the myelin at 12 weeks postinjury—were observed on tissue sections from the both the PRF and control group. Remyelinization, vascular formations, damage in the large-diameter axons, collagen formation, and myelin remnants phagocytosed by macrophages were present in the tissue sections of both groups but more prominent in the PRF treatment group (Fig. 3).

The percentage of axons with optimum g-ratio was significantly lower in the PRF treatment group than the control group ($P = 0.02$; Fig. 4). The mean density of axons was also lower in the PRF treatment group but, although compatible with the g-ratio results, this difference was not statistically significant ($P = 0.139$; Fig. 5).

Electromyography Results

At the preinjury time point, nerve conduction speed was calculated for Δt1, Δt2, Δt3, and Δt4, respectively (Fig. 6). Although the postinjury nerve conduction speeds of the PRF group were slightly higher than those of the control group and slower than the preinjury conduction speeds, there was no statistically significant difference among the groups or time points. In addition, there were no significant differences in amplitude of deflection between the groups or time points (Fig. 7).

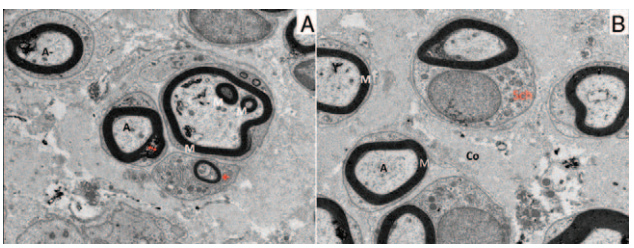

FIGURE 3. Electron microscopy images of nerve tissue. (A) Control group; M: myelinated nerve fiber, A: axon, A-: regression of axon, ⇉: myelin sheath residual in axon's cytoplasm, ↔: dilation of myelin sheath, Co: collagen fiber (uranyl acetate and lead citrate, 6000× magnification). (B) PRF group; M: myelinated nerve fiber, A: axon, ⏭: separation of myelin sheath, Sch: Schwann cell cytoplasm, Co: collagen fiber (uranyl acetate and lead citrate, 6000× magnification). PRF, platelet-rich fibrin.

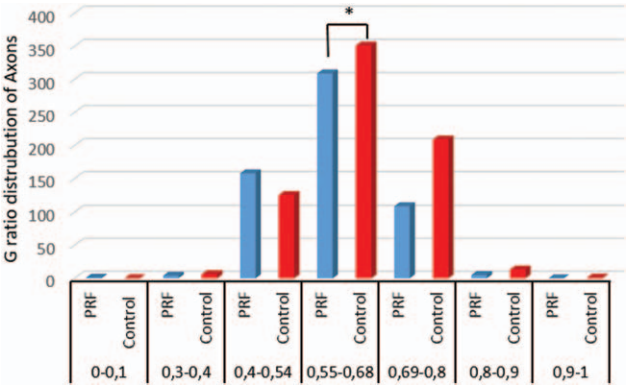

FIGURE 4. G-ratio distribution of evaluated axons. PRF, platelet-rich fibrin.

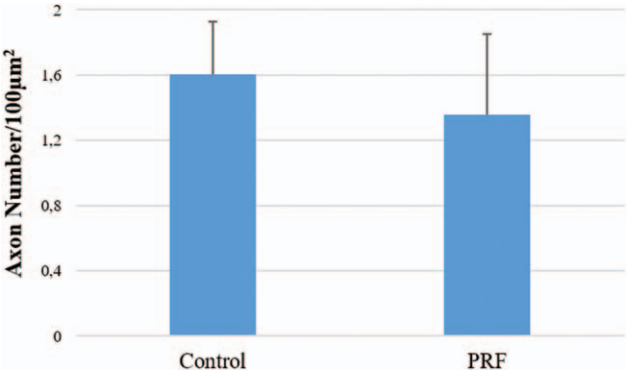

FIGURE 5. Axon density in nerve sections. Mean and SD axon density values for the control and PRF groups at 12 wk postinjury. PRF, platelet-rich fibrin.

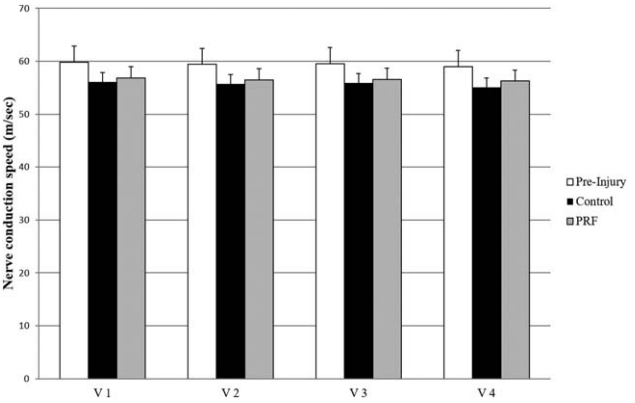

FIGURE 6. Nerve conduction speed. Nerve conduction speeds preinjury and in the control and PRF groups at 12 wk postinjury. PRF, platelet-rich fibrin.

DISCUSSION

Because of previous controversial results, alternative treatment approaches for regenerative nerve repair remain the subject of investigation. Spontaneous healing of nerve fibers is expected to occur within a few weeks or months after damage.[11] In the present study, it was hypothesized that an autologous PRF membrane may have a

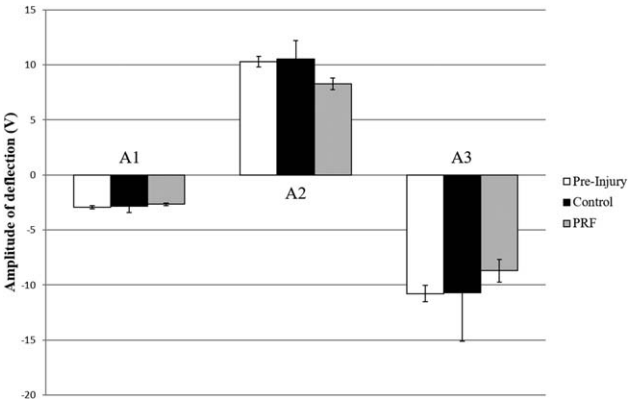

FIGURE 7. Nerve conduction amplitude. Amplitude deflection of impulse preinjury, and in the control and PRF groups at 12 wk postinjury. PRF, platelet-rich fibrin.

tubulization effect and thus improve regeneration capacity by releasing GFs during the spontaneous healing of peripheral nerve crush injury. Although PRF membrane insertion led to excessive fibrotic tissue formation, remyelination, and increased vascularization at the damaged region, it was not able to produce the expected positive effects on axon density and impulse transmission in the present study.

Peripheral nerve repair capacity is the one of the slowest repair capacities in the body.[17] Therefore, a number of techniques aimed at increasing regenerative capacity of peripheral nerves have been investigated, including microsuturing, gluing (fibrin and cyanoacrylate glues), tubulization, grafting (allograft, autograft), and laser welding.[18–23] Additionally, the use of agents such as stem cells, PRP, PRF, and GFs for regenerative purposes has been previously studied[11–15,24,25]; however, none of these techniques has proven to be fully successful in effectively biostimulating nerve tissue.

The histomorphometric assessments of Elgazzar et al[11] and Farrag et al[12] showed significantly higher axon density in the distal segment of reanastomosed nerves that were treated with prepared autologous PRP gel than in nerves on the contralateral side that received no additional treatment. They concluded that PRP significantly enhanced the number of regenerating nerve fibers after neuroanastomosis. Giannesi et al[14] reported that PRP application around neurorrhaphy-type damage could improve nerve regeneration capacity. Increased regeneration was associated with increased levels of bioactive proteins supplied by PRP.[11,14] Bioactive proteins found in platelets, plasma, and white blood cells organize nerve fiber regeneration. Increasing the concentration of these bioactive proteins by adding PRP may stimulate the healing process of damaged nerve fibers.[14]

In the light of previous findings, we chose to investigate the effects of autologous PRF (new generation platelet concentrate) on nerve regeneration. PRF has several advantages over PRP gel, such as shorter duration of generation, easier formation technique under clinical conditions and not requiring any additional heterogeneous agent.[26] As PRP can only be applied in a gel or solution form, a silicon tube is needed for application; PRF, on the other hand, can be applied as an isolated strong membrane. In the present study, a PRF membrane was applied and sutured to the crush injury region, acting as a tube.

It is well known that the tubulization technique enhances the regeneration capacity of nerve damage.[4] Several tubulization techniques, such as decalcified bone, vein conduit, and silicone tube, have been investigated, with favorable effects reported.[2,27]

In the present study, PRF membrane was successfully applied as a tube around the damaged nerve region and stabilized with sutures, but its expected contribution to regeneration was not observed according to histomorphometric and EMG assessment. PRF supplies many important GFs for the healing process, such as PDGF, TGF-β1, and VEGF.[10] The release of these factors may be the reason for prominent neovascularization, increased collagen formation, and stimulated remyelination in our PRF group. Factors such as g-ratio, axon density, and functional recovery did not seem to be sufficient to promote nerve regeneration. PRF membrane was also not able to act as a confidential barrier for collagen fibril infiltration around the damage region; therefore, its tubulization capacity was not effective and reliable. Further and more comprehensive studies should be conducted to clarify these results.

Lichtenfels et al[13] evaluated the functional recovery and histomorphometric regeneration of the sciatic nerve on the ninetieth day after neurotomy in Wistar rats. The authors compared 4 techniques: autologous nerve grafting, silicon tube plus PRF, silicon tube plus PRP, and silicon tube plus saline solution. This study suggested that autologous nerve grafting, as expected, was the best technique when nerve fiber diameter was evaluated via histomorphometric examination; no significant positive effects were observed for PRP and PRF. They also reported that PRP and PRF had significant positive effects on functional nerve recovery. In contrast with Lichtenfels et al's[13] findings, the results of our study suggest that PRF membrane does not have any favorable effects on functional recovery.

Senses et al[15] studied the effects of PRF membrane on transected sciatic nerve. They divided animals into 3 separate groups. Isolated edge-to-edge suturing was performed in the first group, PRF was used as a covering membrane on the sutured region in the second group and the sciatic nerve was transected, sutured leaving a 5 mm gap, and then covered by PRF in the third group. Given that increased neurotrophic factors associated with PRF application may stimulate nerve regeneration, the authors hypothesized that the best results should be expected in the second group. However, they found that PRF membrane covering worsened the histomorphometric results in both the second and third groups.

Our histomorphometric results are consistent with those of Senses et al.[15] Although PRF treatment seemed to increase remyelinization and stimulate cellular proliferation, it significantly worsened g-ratio. PRF membrane may not block the infiltration of collagen into the connective tissue layer of nerves, and this infiltration may explain the worsened histomorphometric results following PRF application. The contribution of PRF membrane to nerve regeneration may be limited to guiding cell proliferation, increasing cell proliferation, and acting as a barrier membrane to undesirable infiltration during the healing process. While PRF membrane stimulates cell proliferation; it did not act as a barrier in a crushed rabbit sciatic nerve model.

Most of the previously reported studies evaluating the regeneration capacity of platelet concentrates on peripheral nerve damage were performed on ruptured sciatic nerves in rat models in the field of oral and maxillofacial surgery.[11,13–15] The advantages of investigating the sciatic nerve are that it enables comparison with previous studies and standardization across the literature. It is necessary to collect 8 to 10 mL of autologous blood to obtain a strong, high-quality PRF membrane; therefore a rabbit model was preferred to a rat model in the present study. Contusion damage (neuropraxia) of peripheral nerves is more common than nerve rupturation (neurometsis) in the field of oral and maxillofacial surgery.[1] Because of this, investigating a crush injury model was chosen for our study to simulate contusion damage. The results of the present study only refer to a 12-week postinjury time point, but it is not known exactly how long nerve regeneration should take. For this reason, further investigation at different stages is required to further support these results.

PRF membrane use has an increasing range of applications in the field of oral and maxillofacial surgery. Controversial results have

been reported regarding the regeneration capacity of platelet concentrates on peripheral nerve injury in recent experimental studies. According to the findings of the present study, even though PRF membrane stimulated vascular formation and remyelination around the injury site, it did not significantly improve either axon density or functional recovery. Nerve regeneration includes very specific cellular conditions and PRF membrane could not make any significant positive effect on this regeneration in rabbits. Nerve specific growth factors containing scaffolds may be applied in future studies.

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The First Patient Report of Tongue Abscess Among Iraqi Population

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Abstract: Tongue abscess is a serious clinical entity which scarcely affects the tongue. It should be treated urgently to prevent airway obstruction or dissemination of infection to a more deep or distant area of the body. This article presented the first clinical report of tongue abscess in Iraq which discussed its clinical presentation, diagnosis, and treatment with a review of literature.

Key Words: Lingual abscess, tongue abscess, tongue enlargement

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Received July 1, 2017.
Accepted for publication October 6, 2017.

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The authors report no conflicts of interest.

ISSN: 1049-2275
DOI: 10.1097/SCS.0000000000004257