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Evaluation of Facial Nerve Regeneration by End- to- End Suturing Technique versus adding a PRF Membrane as a Scaffold in Rats

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ABSTRACT
Background: Paralysis of the facial nerve cause a functional and emotional problems to the patients, treatment methods need to be improved.
Materials and Methods: Twenty-four Sprague Dawely rats with average weight of (250---300 g), were devided randomly into two groups, group 1 (control) the facial nerve was transected and left without any treatment, group 2 (PRF) the facial nerve transected then the two ends of the nerve sutured with (8-0) suture then a PRF membrane was wrapped over the sutured area.
Results:PRF group showed better bundles organization with no adhesion with surrounding inflamed area **compared to** cutting group.
Aim of the study: The aim of this study was the evaluation for the effects of PRF on peripheral nerve regeneration in the facial nerve of rats by using histopathologic analyses.
Conclusion: Our findings suggested that PRF therapy may be promising approach for peripheral nerve cut injury.
Key Words: Nerve regeneration, PRF, rat, facial nerve.

I. INTRODUCTION:

Following head and neck surgery, cranial nerve damage may result in permanently disabled function.The facial nerve (CN VII) and the recurrent laryngeal nerve are two of the most often damaged nerves (RLN, CN X). Congenital, infectious, idiopathic, traumatic, neoplastic, endocrine, neurologic, and systemic conditions can all result in theseinjuries.

Facial expressions are made possible by the facialnerve, which provides nerveimpulses to the facial muscles,[1] The physical and mental effects of facial paralysis are devastating. Facial nerve paralysis can have a wide variety of origins, including medical procedures, infections, accidents, birth defects speech and articulation difficulties, aesthetic impairments, and the inability to communicate emotions through facial muscle are among the most clinically significant effects of inadequate eye closure.[2]

Nerve injuries can sometimes happen during oral and maxillofacial surgeries like orthognathic surgery, dentoalveolarsurgery, temporomandibular joint surgery, cyst enucleation, removal of the third molar, implant placement, and anaesthesia injections.Temporary inferior alveolar nerve (IAN) injuries happen between 0.5% and 5% of the time, while permanent injuries happen less than 1% of the time. Permanent injuries can cause numbness in the lower teeth, chin, and lower lip, trouble speaking and chewing, drooling of fluids and saliva, and allodynia.[3, 4]

This may have a significant impact on the quality of life of patients and lead to psychological stress for patients and their family.[5, 6]Therefore, the facial nerve defect repair is the subject of the present research.

Materials and methods: Twenty-four Sprague Dawely rats with average weight of (250---300 g), were devided randomly into two groups
Group 1 (cut group):The rats received facialnerve transection, then the facial nerveendings were approximated and allowed to recover spontaneously.
Group 2: anastomosis was done using a combined method byapproximation of the two ends of the nerve with suturingusing(8-0 Trulene sutures)and aPRF membrane applied all around to cover the sutured area.
Platelet rich fibrin preparation: Blood samples were collected from another rat to be a blood donor. 5-6 milliliters of blood was obtained in a sterile tube, without adding anticoagulant, then the tubes were placed in a centrifug machine for 10

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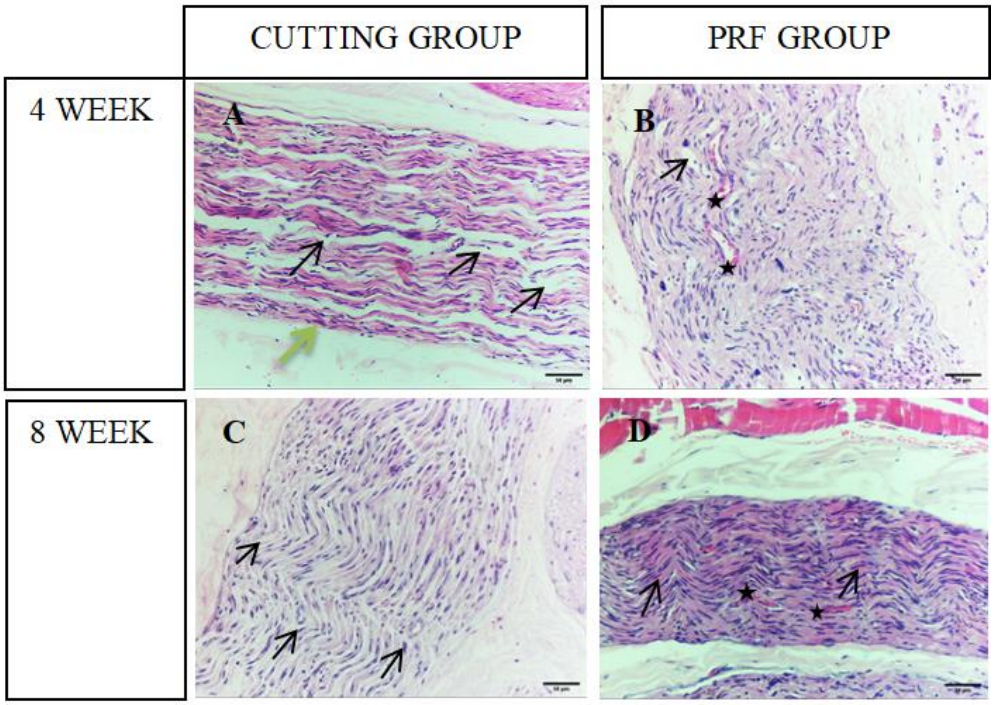
minutes at 3000 rpm, then The blood was separated into three layers: on the top acellular plasma, in the middle the PRF clot, and at the bottom of the tubes the red blood cells, After centrifugation, the PRF clot was removed from the tube using sterile tweezers, and placed over a sterile glass slap to be pressed and formed into thin membrane. (Many experimental study on plasma transfusion from rat to another rat was done)[7].

II. RESULTS:

Haematoxyline and Eosin (H&E) Staining Procedure

The sections were immersed in filtered Harris Hematoxylin for 1 min, rinsed with tap water and water exchanged until it was clear. Then, the sections were immersed in eosin stain for 1-2 min. One more time, They were rinsing with tap water and exchanging water till the water was clear. They were dehydrated in ascending alcohol solutions (50%, 70%, 80%, 95%, 100%) and then xylene was used to remove any remaining moisture. Lastly, the cover slip was mounted with each mount onto a marked glass slide.[8]



Longitudinal sections of the facial nerve following cut and repair at different time points (H&E). A (cutting only group after 4 weeks) (black arrows) pointed to demyelinated nerve fibers, B (PRF group after 4 weeks) star pointing to showing blood vessels, C (cutting only group after 8 weeks) showing nerve fibers more organized , F (PRF group after 8 weeks) showing alignment and organization of nerve fibers Original magnification ×200 and scale bar

Longitudinal sections of the facial nerve following cut and repair at different time points (H&E). A (cutting only group after 4 weeks) showing inorganization, vacuolation(black arrows) and segmental demyelinated nerve fibers, low number of proliferated and activated Schwann cells. B (PRF group after 4 weeks) showing better organization and alignment with more newly generated blood vessels (star). C (cutting only group after 8 weeks) showing better organization and parallelism with minimal disorientation of the fibers. D (PRF group after 8 weeks) showing perfect organization (black arrow) and alignment with more newly generated blood vessels(stars) and no vacuolation or demyelination.

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III. DISCUSSION

Trauma and surgical procedures can cause damage to the facial nerves, without medical intervention (surgery or other biological agents), nerve injury has a poor prognosis, and functional issues have a major impact on quality of life.[9, 10]many approaches have been tried for treatment (repair).

Facial nerve axotomy (FNA) tried to be repaired in a number of ways, but there is still no agreement on the best strategy for regenerating the nerve.[11]the present study examined the healing process of the facial nerve after it has been cut, and we illustrated how the application of platelet-rich fibrin affects the healing process when use a PRF membrane as a scaffold.

The common anastomosing technique is microsurgical suturing, which requires highly technical skills. Other anastomotic techniques have been used, including gluing the nerve endings with biological (fibrin) or cyanoacrylate glue and direct laser soldering, but each method has its drawbacks. suturing the outer epineurium is the simplest suturing method as it reduce the scarring.[12]
Additionally regeneration of damaged nerves can be improved by using the tublization technique as is widely known. researchers have shown success using a variety of tublization methods, including decalcified bone, venous conduit, and silicon tube.[13-15]

Platelet-rich concentrate is an autologous concentration of platelets and growth factors, the platelet rich plasma (PRP) and platelet rich fibrin (PRF), also the platelet rich growth factor (PRGF) can be extracted from the normal fresh blood with different preparation methods, There are a number of advantages that PRGF and PRF are reported to have over PRP.[16-18]

In spite of the large number of research that have been conducted on the efficiency of PRP on nerve regeneration, the number of studies that have been conducted on PRF has been rather low**.**

Variations in platelet and leukocyte content, polymerization method, and fibrin architecture further characterize these biomaterials. Polymerization of plasma yields PRGF, which consists of platelets at a concentration 2–3 times greater than in normal blood.[19]

This study showed better histological outcome with increase mylinated fibers and better organization of nerve fibers, this might be due to the fact that PRF is generated in way that closely mimics natural process through the centrifugation of blood without the use of any external agent.[20, 21]

Concomitantlyresultsof other experimental models used platelet-rich plasma (PRP) as a platelet concentrate that stimulate the nerve generation.

Additionally**Ding XG et al** .[22] found that the group treated with PRP showed a statistically significant increase in the number of myelinated axons compared with the injured control group.

In the present study we combined epineural suture repair,with PRF membrane as a nerve wrap, reduced intraneural scar formation, adhesion, during the initial phase of regeneration and showed better histological result of the PRF group over cutting group.

It is hypothesized that an inflammatory barrier, such as that provided by a nerve wrap around the nerve cut area, may suppress this reaction and promote axonal regeneration.[23, 24]

The results of the present studyin agreement with**Kim et al**.[25] who described a reduction in intraneural connective tissue after applying type 1 bovine collagen wraps to rat sciatic nerve repairs.

Senses et al .[16] were examined PRF membrane effects on transected sciatic nerve (both in edge to edge cut, 5 mm gap), They found that PRF may enhance regeneration of the nerves due to enhanced neurotrophic factors.

Lichtenfels et al.[17] shown the efficacy of PRP and PRF in facilitating functional recovery during nerve regeneration, despite the fact that histomorphometric study did not support their results. In this study, it showed better histological outcome when we added the PRF to the injured nerve than the result of Lichtenfels et al.
Conclusion: PRF as a wrap and anti-inflammatory barrier reveled better results, further functional and histopathological analysis are nedded to verify this effect.

REFERENCE

[1]. Toulgoat F, Sarrazin JL, Benoudiba F, Pereon Y, Auffray-Calvier E, Daumas-Duport B, Lintia-Gaultier A, Desal HA. Facial nerve: from anatomy to pathology. Diagn Interv Imaging 2013; **94**: 1033-1042.

[2]. Hadlock TA, Greenfield LJ, Wernick-Robinson M, Cheney ML. Multimodality approach to management of the paralyzed face. Laryngoscope 2006; **116**: 1385-1389.

[3]. Yadav SK, Sachdeva A, Verma AK. Inferior alveolar nerve damage following removal of mandibular third molar teeth In: Proceedings of the., Year.

[4]. Genú PR, Vasconcelos BC. Influence of the tooth section technique in alveolar

nerve damage after surgery of impacted lower third molars. Int J Oral Maxillofac Surg 2008; **37**: 923-928.

[5]. Lloyd BM, Luginbuhl RD, Brenner MJ, Rocque BG, Tung TH, Myckatyn TM, Hunter DA, Mackinnon SE, Borschel GH. Use of motor nerve material in peripheral nerve repair with conduits. Microsurgery 2007; **27**: 138-145.

[6]. Ignatiadis IA, Yiannakopoulos CK, Barbitsioti AD, Avram AM, Patralexis HG, Tsolakis CK, Papalois AE, Xenakis TH, Beris AE, Soucacos PN. Diverse types of epineural conduits for bridging short nerve defects. An experimental study in the rabbit. Microsurgery 2007; **27**: 98-104.

[7]. Liu A, Guo E, Yang J, Yang Y, Liu S, Jiang X, Hu Q, Dirsch O, Dahmen U, Zhang C, Gewirtz DA, Fang H. Young plasma reverses age-dependent alterations in hepatic function through the restoration of autophagy. Aging Cell 2018; **17**: e12708.

[8]. Choukroun J, Adda F, Schoeffler C, Vervelle A. Une opportunit?? en paro-implantologie: Le PRF. Implantodontie 2001; **42**: 55-62.

[9]. Lucena EE, Guzen FP, Cavalcanti JR, Marinho MJ, Pereira WO, Barboza CA, Costa MS, do Nascimento Júnior ES, Cavalcante JS. Plasticity of mesenchymal stem cells from mouse bone marrow in the presence of conditioned medium of the facial nerve and fibroblast growth factor-2. ScientificWorldJournal 2014; **2014**: 457380.

[10]. Bastami F, Vares P, Khojasteh A, Khojasteh A. Healing Effects of Platelet-Rich Plasma on Peripheral Nerve Injuries. The Journal of craniofacial surgery 2017; **28**: e49-e57.

[11]. Haggag MA, Salem AE-s, Elsherbini AM. Sustained Release In Situ Thermogelling Hydrogel of Cerebrolysin for Treatment of Facial Nerve Axotomy in Rats. Journal of Oral and Maxillofacial Surgery 2022; **80**: 949-959.

[12]. Edshage S. PERIPHERAL NERVE SUTURE. A TECHNIQUE FOR IMPROVED INTRANEURAL TOPOGRAPHY. EVALUATION OF SOME SUTURE MATERIALS. Acta Chir Scand Suppl 1964; **15**: Suppl 331:331+.

[13]. Battiston B, Geuna S, Ferrero M, Tos P. Nerve repair by means of tubulization: literature review and personal clinical experience comparing biological and synthetic conduits for sensory nerve repair. Microsurgery 2005; **25**: 258-267.

[14]. Bastos Dos Santos E, Fernandes M, Gomes Dos Santos JB, Mattioli Leite V, Valente SG, Faloppa F. Study of tibial nerve regeneration in Wistar rats in primary neurorrhaphy with and without gap, wrapped in vein segments. Acta Ortop Bras 2012; **20**: 165-169.

[15]. Heijke GC, Klopper PJ, Dutrieux RP. Vein graft conduits versus conventional suturing in peripheral nerve reconstructions. Microsurgery 1993; **14**: 584-588.

[16]. Şenses F, Önder ME, Koçyiğit ID, Kul O, Aydin G, Inal E, Atil F, Tekin U. Effect of Platelet-Rich Fibrin on Peripheral Nerve Regeneration. J Craniofac Surg 2016; **27**: 1759-1764.

[17]. Lichtenfels M, Colomé L, Sebben AD, Braga-Silva J. Effect of Platelet Rich Plasma and Platelet Rich Fibrin on sciatic nerve regeneration in a rat model. Microsurgery 2013; **33**: 383-390.

[18]. Ikumi A, Hara Y, Yoshioka T, Kanamori A, Yamazaki M. Effect of local administration of platelet-rich plasma (PRP) on peripheral nerve regeneration: An experimental study in the rabbit model. Microsurgery 2018; **38**: 300-309.

[19]. Anitua E, Sánchez M, Nurden AT, Nurden P, Orive G, Andía I. New insights into and novel applications for platelet-rich fibrin therapies. Trends Biotechnol 2006; **24**: 227-234.

[20]. CHOUKROUN J, ADDA F, SCHOEFFLER C, VERVELLE A. Une Opportunité en Paro-implantologie Le PRF:(Platelet Rich Fibrin).

[21]. Kang YH, Jeon SH, Park JY, Chung JH, Choung YH, Choung HW, Kim ES, Choung PH. Platelet-rich fibrin is a Bioscaffold and reservoir of growth factors for tissue regeneration. Tissue Eng Part A 2011; **17**: 349-359.

[22]. Ding XG, Li SW, Zheng XM, Hu LQ, Hu WL, Luo Y. The effect of platelet-rich plasma on cavernous nerve regeneration in a rat model. Asian J Androl 2009; **11**: 215-221.

[23]. Kokkalis ZT, Pu C, Small GA, Weiser RW, Venouziou AI, Sotereanos DG.

Assessment of processed porcine extracellular matrix as a protective barrier in a rabbit nerve wrap model. J Reconstr Microsurg 2011; **27**: 19-28.

[24]. Lundborg G, Rydevik B. Effects of stretching the tibial nerve of the rabbit. A preliminary study of the intraneural circulation and the barrier function of the perineurium. J Bone Joint Surg Br 1973; **55**: 390-401.

[25]. Kim PD, Hayes A, Amin F, Akelina Y, Hays AP, Rosenwasser MP. Collagen nerve protector in rat sciatic nerve repair: A morphometric and histological analysis. Microsurgery 2010; **30**: 392-396.