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Stimulation of morphofunctional repair of the facial nerve with photobiomodulation, using the end-to-side technique or a new heterologous fibrin sealant

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ABSTRACT

This research evaluated the influence of Photobiomodulation Therapy (PBMT) on lesions of the facial nerve repaired with the end-to-side technique or coaptation with a new heterologous fibrin sealant. Thirty-two Wistar rats were separated into 5 groups: Control group (CG), where the buccal branch of the facial nerve was collected; Experimental Suture Group (ESG) and Experimental Fibrin Group (EFG), in which the buccal branch was end-to-side sutured to the zygomatic branch on the right side of the face or coaptated with fibrin sealant on the left side; Experimental Suture Laser Group (ESLG) and Experimental Fibrin Laser Group (EFLG), in which the same procedures were performed as the ESG and EFG, associated with PBMT (wavelength of 830 nm, energy density 6.2 J/cm^2, power output 30 mW, beam area of 0.116 cm^2, power density 0.26 W/cm^2, total energy per session 2.16 J, cumulative dose of 34.56 J). The laser was applied for 24 s/site at 3 points on the skin's surface, for a total application time of 72 s, performed immediately after surgery and 3 times a week for 5 weeks. A statistically significant difference was observed in the fiber nerve area between the EFG and EFLG (57.49 ± 3.13 and 62.52 ± 3.56 μm^2, respectively). For the area of the axon, fiber diameter, axon diameter, myelin sheath area and myelin sheath thickness no statistically significant differences were found ($p < 0.05$). The functional recovery of whisker movement occurred faster in the ESLG and EFLG, which were associated with PBMT, with results closer to the CG. Therefore, PBMT accelerated morphological and functional nerve repair in both techniques.

1. Introduction

Nerve injuries may occur due to several types of trauma, such as lacerations with sharp objects, gunshot wounds, crushing, fractures, tumor resections, orthognathic surgeries, and motor vehicle accidents [1–5]. Damage to the facial nerve (FN) can lead to functional alterations such as asymmetry and loss of facial expression, causing disorders related to self-image and recognition of the individual, as well as mental health alterations, suffering, and social isolation [4,6–9].

When the distal and proximal stumps of the damaged nerve are isolated and distant, it is difficult to apply the end-to-end neurorrhaphy technique, which is considered the gold standard [9,10], so an alternative technique may be used, the end-to-side neurorrhaphy [11]. This repair technique is based on the use of a graft with donor nerves that, laterally connected to the nerve to be recovered, provides axonal sprouting of the intact nerve to the receiver nerve. This technique may be applied to different nerves, including the facial nerve [3,12–16].

Aiming to establish an environment that is less invasive than traditional repair suturing (the gold standard), new surgical adhesives has been created, such as collagen, fibrin glue, cyanoacrylate, and gelatin.

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The new heterologous fibrin sealant (NHFS) is extracted from snake venom (*Crotalus durissus terrificus*) and, because it does not contain human blood, it avoids the possible transmission of infectious diseases. It is a clinically useful tool due to its flexibility and variety of applications, demonstrating a good bond strength and biocompatibility, having an important role in cell and tissue regeneration process [17–26].

Therapy using physical methods such as therapeutic ultrasound and photobiomudalation therapy (PBMT) may provide the benefits of faster functional and nerve recovery [27–32]. PBMT demonstrates therapeutic effects such as local analgesia, antiedema action, anti-inflammatory and healing actions, and tissue biostimulatory effects that may provide an increase in the speed of growth of sectioned nerves [27,33].

The photostimulatory effect of PBMT refers to its absorption by the tissues through photoreceptors, facilitating mitochondrial respiration, creation of adenosine triphosphate (ATP), additional transportation of Ca^{2+} in the cytoplasm, and the beginning of conducted pathways mediated by reactive oxygen species (ROS), cyclic adenosine monophosphate (AMP) and nitric oxide. These effects lead to stimulation of various transcription factors related to migration and cell proliferation, promoting tissue repair and regeneration [27,34,35]. The transcription factors NF-kB and HIF-1α NRF2 can be activated, causing a decrease in the inflammatory response, playing an important role in the repair process [36–40].

The performance of low-level lasers associated with the use of the NHFS in end-to-side repair of damaged facial nerves is still not established, especially in anatomical and functional aspects. In view of this, the objective of the current study was to evaluate the influence of PBMT on damaged terminal branches of the FN repaired through the end-to-side technique with epineural suture or coaptation with the NHFS.

2. Materials and Methods

2.1. Animals

The experimental research procedures were approved by the Ethics Committee on Research in Animals of the Bauru School of Dentistry, University of São Paulo (São Paulo, Brazil), with the Protocol number 007/2013.

Thirty-two male Wistar rats (*Rattus norvegicus*) were used. They were 80 days old and the average weight was 250 g. The animals remained in appropriate boxes with no movement restrictions and received water and food ad libitum. They were exposed to an approximate temperature of 22 °C, with a 12-hour regime of light and dark, obtained by use of a timer in the animal maintenance room.

2.2. Experimental Groups

The animals were randomly divided into 1 control group (CG) and 4 experimental groups (EG), as described below:

1. Control group (CG): Consisting of 8 animals that were not submitted to surgical procedures or PBMT, where the buccal branch of the facial nerve (BBFN) was dissected and collected for histomorphological and histomorphometric analysis.
2. Experimental suture group (ESG) and experimental fibrin group (EFG): Consisting of 12 animals in which the BBFN was sectioned on both sides of the face, but on the right side, an end-to-side graft suture was used to connect the distal stump of the buccal branch (dsBB) to the zygomatic branch of the facial nerve (ZBFN), whereas on the left side, the same procedures were performed, using the NHFS for coaptation.
3. Experimental suture laser group (ESLG) and experimental fibrin laser group (EFLG) associated with PBMT: Consisting of 12 animals that underwent the same surgical procedures as those in the ESG and EFG, in combination with PBMT.

2.3. New Heterologous Fibrin Sealant

Venom from *Crotalus durissus terrificus* snakes was milked at State University of São Paulo (UNESP), more specifically Center for the Study of Venoms and Venomous Animals (CEVAP) and pooled according to good manufacturing practices (GMP). All the snakes are microchipped to ensure the traceability of the venom lots used in the composition of heterologous fibrin sealant. After filtration and lyophilization, the venoms are stored in the Venoms Bank of CEVAP. Cryoprecipitate is the insoluble fraction, a cold precipitate of frozen fresh plasma (FFP) from *Bubalus bubalis* buffalos. It contains fibrinogen, factor VIII (F VIII), Willebrand factor (F vW), factor XIII (F XIII) and fibronectin.

It must contain at least 80 units of factor VIII and between 150 and 250 mg of fibrinogen. Each unit has a volume from 10 to 20 ml, which must be stored at − 20 °C and has a shelf life of one year. In sum, the cryoprecipitate extracted to be applied as a new heterologous fibrin sealant standardized by CEVAP is a product that is safe and free from undesirable substances. The formulation, as well as its storage, handling and dosage are described in detail in the internationally required patents (PCT/BR2015/000065 and PCT/BR2015/000064) [17,18,21,22].

At the time in experimental surgery, the components were first thawed, then reconstituted, mixed, and applied according to the following protocol: The first bottle (Eppendorf®) contained fibrinogen obtained from buffalo blood (5 µl); the second contained calcium chloride (2 µl); and the last bottle contained a thrombin-like fraction (1 µl); for a total of 8 µl. The production process was described in Ferreira Junior et al. [41].

2.4. Surgical Procedures

To perform the surgical procedures, at 80 days old, all of the animals in the experimental groups (ESG, EFG, ESLG and EFLG) were weighed and underwent general anesthesia through an intramuscular injection of the anesthetic Zoletil 50® (Virbac of Brazil), which contains a 1:1 ratio (125:125 mg) of tiletamine hydrochloride and zolazepam hydrochloride (0.15 ml/kg/IM). Then, the animals were submitted to asepsis, trichotomy and positioning of the animal in the lateral decubitus position.

Initially an incision was made on the face with a No. 15 scalpel blade (approximately from the tragus of the ear toward the labial commissure), with subsequent incision to expose the BBFN, which was sectioned with straight-tip scissors (Fig. 1A) without removing fragments observed on the surgical microscope (DFV, Brazil). The proximal stump of the BBFN was positioned end-to-side to the ZBFN.

In the ESG, an end-to-side epineural suture with 10-0 nylon monofilament (Ethicon®, Johnson & Johnson, Brazil) was performed on the right side of the face. In the EFG, the nerve stumps were approximated and coaptation with the NHFS (Fig. 1B) on the left side of the face. The surgery was finished with skin sutures using 4-0 silk thread (Ethicon®, Johnson & Johnson, Brazil).

The same surgical procedures were performed on the ESLG and EFLG animals, adding PBMT.

2.5. Photobiomodulation Therapy

In groups ESLG and EFLG, photobiomodulation therapy (PBMT) was applied to the surgical sites on the animals with a continuous pulse using a gallium-aluminum-arsenide laser (GaAlAs, Laserpulse IBRAMED®, Brazil). The protocol used was: wavelength of 830 nm, energy density of 6.2 J/cm^2, with optical power output potency of 30 mW, power density of 0.26 W/cm^2, beam area of 0.116 cm^2, total energy per session of 2.16 J and cumulative dose of 34.56 J. Each laser application session was applied for 24 s/site at 3 points in the perpendicular surgical area on the skin's surface, for a total application