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SciVerse ScienceDirect
Journal of Prosthodontic Research 57 (2013) 93–98
Journal of Prosthodontic Research
www.elsevier.com/locate/jpor

Original article

Low-intensity pulsed ultrasound enhances palatal mucosa wound healing in rats

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Received 30 January 2012; received in revised form 30 October 2012; accepted 16 November 2012
Available online 24 December 2012

Abstract

Purpose: Low-intensity pulsed ultrasound (LIPUS) has been used in fracture treatment to shorten the time needed for biological wound healing. However, the influence of LIPUS exposure on oral wound healing has not been sufficiently investigated. This study was conducted to evaluate low-intensity pulsed ultrasound on wound healing in palatal excisional wounds of rats.
Methods: Excisional wounds, 5 mm in diameter, were made in the center of the palate of rats. Animals were divided into four experimental and control groups (1-week after LIPUS exposure, 1-week control, 2-week after LIPUS exposure, and 2-week control). The affected area in the experimental group was exposed to LIPUS, daily frequency: 3 MHz, intensity: 160 mW, exposure time: 15 min. Specimens were fixed in 10% neutral formalin solution immediately after sacrifice. The wound was measured histologically.
Results: Wound width in the LIPUS group tended to be smaller than that of the control group. The experimental group in both 1-week and 2-week groups showed that unhealed areas were significantly smaller by LIPUS than those in the control groups ($P < 0.05$).
Conclusion: Our results suggest that the use of LIPUS on palatal excisional wounds was effective in promoting epithelial and connective tissue closure.

Keywords: Low-intensity pulsed ultrasound; Palatal mucosa; Wound healing

1. Introduction

Free gingival grafts have been used as a predictable and reproducible technique for increasing the width of keratinized gingiva around natural teeth [1] and implants [2–4]. Post-operative morbidity associated with an open secondary wound site includes inconvenience caused by pain and post-surgical bleeding [5,6]. The necessity for enhancements in healing times arises when the palatal donor site is required for repeated procedures [7]. This is especially important in certain wound types, such as those created at palatal donor sites used for free soft tissue grafts, where a significant amount of tissue loss occurs [8]. These are often associated with post-operative discomfort, pain, and sometimes delayed healing [9].

On the other hand, physical therapy to promote healing by applying physical energy to wounds has been attracting attention [10]. Physical therapy represents medical care utilizing the physical actions of heat, electricity, light, X-rays, air, and hot springs. Energies used are roughly divided into electromagnetic [11] and mechanical energies [12]. A mechanical energy attracting attention over the last 10 or more years is ultrasound therapy.

Physical medicine employing low-intensity pulsed ultrasound exposure was initiated in reports from Buchtala et al. [13] and Maintz et al. [14] in the 1950s, in which low-intensity ultrasound promoted the fracture healing process, and Pilla et al. reported an increase in the mechanical strength in a tibial fracture model in vivo [15]. In an in vitro study, Yang et al. reported an increase in aggrecan mRNA in a rat femoral

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1883-1958/$ – see front matter
http://dx.doi.org/10.1016/j.jpor.2012.11.001

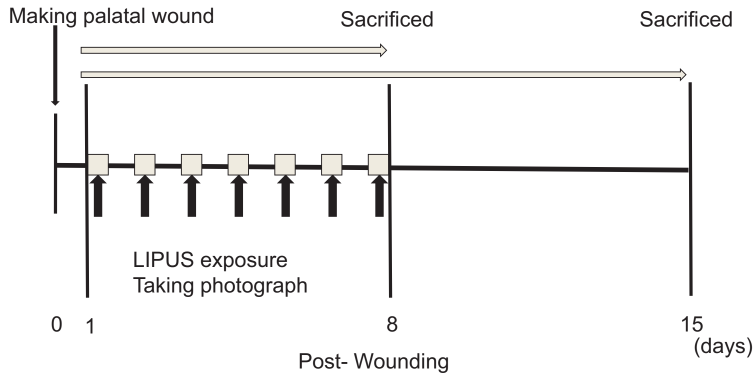

Fig. 1. Timeline of the experiment. General anesthesia was administrated daily for LIPUS exposure and taking of photographs.

fracture model [16], Sant et al. reported that LIPUS exposure led to the up-regulation of interleukin-8, basic fibroblast growth factor, transforming growth factor-β, and alkaline phosphatase [17], and the mechanism has been elucidated with regard to fracture healing and clinically applied for the promotion of fracture healing in the orthopedic field. It is also utilized for the promotion of healing after tooth extraction [18], implant placement [19], and orthodontic tooth transposition in the dental field [20], and the effectiveness of LIPUS exposure has been reported. Regarding wound healing of soft tissue, we previously reported that connective tissue growth factor (CTGF) expression in gingival cells (GE1) was enhanced by LIPUS exposure, promoting soft tissue healing [21]. However, only a single in vivo study performed by Ikai et al. has been reported in which Hsp70 expression was enhanced by LIPUS exposure after flap surgery [22]. The influence of LIPUS exposure on healing of soft tissue wounds has not been sufficiently investigated.

In this study, to clarify the wound healing-promoting effects of LIPUS exposure, a palatal excisional wound experimentally prepared in the rat palatal mucosa was exposed to LIPUS and histopathologically investigated.

2. Materials and methods

Twenty-eight male Wistar rats at 13 weeks of age and weighing 300–450 g were used. Animals were individually housed in cages and maintained under a lighting cycle with lights on between 8:45 and 20:45. Animals were given free access to drinking water (tap water) and food under free movement conditions.

After one-week preliminary maintenance, animals were anesthetized by intraperitoneal injection of pentobarbital sodium (45 mg/kg, Animal Health Co., USA), restrained with their mouth open, and a wound reaching the bone surface was made in the median palate using a biopsy punch with a 5 mm diameter. Hemorrhage was stopped by compression after wound preparation. Rats were then randomly divided into 4 groups (1-week after LIPUS exposure, 1-week control, 2-weeks after LIPUS exposure, and 2-week control). A LIPUS probe was placed in the rat oral cavity from the day following wound preparation, and the wound was exposed to LIPUS (frequency:

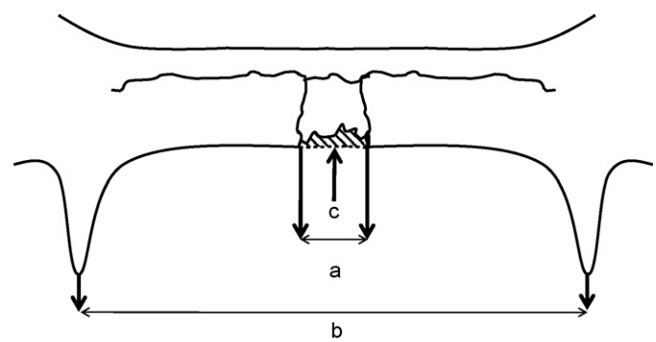

Fig. 2. Reference point for histomorphometric parameters. (a) The distance between the epithelial margins. (b) The palatal width at the examined cut section. (c) To measure and compare the wound area, a virtual line assuming mucosal healing was drawn and the mucosa-defective area in the region was measured.

3 MHz, intensity: 160 mW, exposure time: 15 min) daily for one week (Fig. 1). In the control groups, a probe was placed but no exposure was performed. General anesthesia was administrated daily for LIPUS exposure and taking of photographs. Animals were sacrificed 1 or 2 weeks after completion of LIPUS exposure. Specimens were fixed in 10% neutral formalin solution immediately after sacrifice. Formalin-fixed tissues were dehydrated and embedded in paraffin. Samples were prepared perpendicular to the palatal midline at the greatest diameter of the wound. Four to five-μm sections of tissue were stained with hematoxylin and eosin. Preparations were observed under a light microscope (BZ-9000, Keyence, Osaka). In this study, standardized digital photographs were magnified by a computer, the boundaries of the wound were determined on the magnified image, and surface areas were then calculated using the image analysis software, imageJ (version1.43, National Institute of Health, USA).

To investigate palatal mucosal wound healing, the ratio of the distance between the epithelial margins to the palatal width at the examined section cut was measured. To measure and compare the wound area, a virtual line assuming mucosal healing was drawn and the mucosa-defective area in the region was measured (Fig. 2). All measurements were performed by one implantologist using identical methods in all animals.