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**ORIGINAL ARTICLE**

# A comparative study of wound healing following incision with a scalpel, diode laser or Er,Cr:YSGG laser in guinea pig oral mucosa: A histological and immunohistochemical analysis

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**Abstract**

***Objective.*** This study compared wound healing following incisions with either a scalpel, a diode laser or an Er,Cr:YSGG laser in guinea pig oral mucosa. ***Material and methods.*** Three types of wound were made randomly with either a stainless-steel scalpel, a diode laser or an Er,Cr:YSGG laser in the buccal mucosa of 24 guinea pigs. Five guinea pigs were sacrificed on each of Days 1, 3, 5 and 7 post-surgery. Four guinea pigs were sacrificed on Day 14 post-surgery. Biopsy samples from each oral mucosa wound were examined using light microscopy and the expression of tumor necrosis factor (TNF)-α and transforming growth factor (TGF)-β1 was determined by immunohistochemical staining. The expression of TNF-α and TGF-β1 was evaluated by calculating the percentage of positively stained cells and immunostaining intensity was evaluated using a scale ranging from 0 to 3. ***Results.*** Infiltration of inflammatory cells decreased rapidly at Day 5 post-surgery in all three groups of animals. The highest level of TNF-α expression was found at Day 1 post-surgery for the diode laser wounds. The intensity of TNF-α immunostaining was highest at Day 3 post-surgery and lowest at Day 7 post-surgery for all three groups of animals. For the scalpel wounds, a lower level of TGF-β1 expression was seen until Day 3 post-surgery and a higher level from Day 7 post-surgery compared to laser wounds. The intensity of TGF-β1 immunostaining was highest at Day 1 post-surgery for the diode laser wounds. ***Conclusions.*** The diode laser is considered a good cutting device for oral mucosa; however, more tissue damage occurs than with the use of a scalpel or an Er,Cr:YSGG laser. Larger studies will be needed before fully endorsing the widespread use of the diode laser.

**Key Words:** *Transforming growth factor-β1, tumor necrosis factor-α*

## Introduction

Various surgical instruments used for cutting of oral mucosa have been compared for speed and ease of incision, degree of hemostasis and charring, acute soft tissue injury, pain, swelling and wound healing rates [1–4]. For soft tissue surgery, scalpels and a conventional electrosurgery unit are the instruments of choice. In addition, lasers are an alternative to conventional surgical systems. Scalpels have been used for many years because of their ease of use, accuracy and minimal damage to the surrounding tissue. However, scalpels cannot provide the hemostasis that is helpful when used for highly vascular tissue. The purported advantages of using lasers for soft tissue surgery include coagulation (and therefore a relatively bloodless surgery), sterilization of the surgical site, minimal swelling and scarring, minimal or no suturing and significantly reduced post-surgical pain. However, all laser–tissue interactions produce some degree of tissue vaporization and thermal necrosis of collateral tissues [5,6]. To minimize unwanted thermal damage, different kinds of laser have been investigated in recent years. The efficiency of the laser is based on peak absorption rates of unique laser wavelengths by target material resident in hard/soft tissue and other dental material (e.g. hemoglobin, water, hydroxyapatite, etc.). Certain laser wavelengths (diode laser, etc.) have an affinity for red-pigmented structures, which makes them particularly effective for soft tissue. Other lasers (erbium laser, etc.) have an affinity for water, which makes them

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particularly effective for hard tissue. Some researchers have reported that a diode laser facilitates considerable bacterial elimination, especially of *Aggregatibacter actimomycetemcomitans,* from periodontal pockets [7]. However, due to thermal-induced damage, the laser, as compared to the scalpel, tends to produce more pronounced tissue change. Such changes are associated with an increased inflammatory response and an initial delay in healing response [5,8]. With the recent development of solid-state lasers, a new type of erbium laser, the Er,Cr:YSGG (erbium, chromium: yttrium, scandium, gallium, garnet), has been developed with an emission wavelength of 2.78 μm. Energy from this laser is effectively absorbed by tissue and water with minimal collateral thermal damage and tissue charring. Preliminary investigations have shown that the Er,Cr:YSGG laser can provide efficient and precise ablation for both hard and soft tissues [9,10]. Although a number of ablative-laser techniques have been successfully developed and used in clinical settings, the biomolecular processes influencing wound healing after exposure to laser energy are not well elucidated.

Proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, play important roles in the mediation of inflammation. The quantitative analysis of proinflammatory cytokines in wound extracts can contribute to the determination of vitality and wound age, in particular in the very early post-traumatic interval [11–13]. Transforming growth factor (TGF)-β is composed of a family of multifunctional polypeptide growth factors involved in embryogenesis (cell growth and differentiation), inflammation, regulation of immune response, cell adhesion and migration, extracellular matrix formation, angiogenesis and wound healing [14]. This study was undertaken to compare wound healing following incision with either a scalpel, a diode laser or an Er,Cr:YSGG laser in guinea pig oral mucosa, by assessing the histology and immunohistochemistry (TNF-α and TGF-β1) of wounds.

## Material and methods

All of the surgical interventions and pre- and post-surgical animal care were provided in accordance with the Laboratory Animals Welfare Act, the Guide for the Care and Use of Laboratory Animals and the Guidelines and Policies for Survival Surgery provided by the Institutional Animal Care and Use Committee of the College of Medicine, Catholic University of Korea (CUMC-2008-0120-01).

### *Surgical procedures*

Twenty-four male guinea pigs weighing 450–500 g were used in this study. All guinea pigs were examined by a veterinarian for health status before participation in the study and were kept in individual metal cages at room temperature. The animals received a standard pellet laboratory diet and water. An accommodation time of 2 weeks prior to any kind of surgery was allowed. The guinea pigs were anaesthetized with a mixture (1:8) of xylazine hydrochloride (Rompun; Bayer in Korea, Seoul, South Korea) and ketamine (Ketalar; Yuhan, Seoul, South Korea) which was administered slowly by intraperitoneal injection at a dose of 0.5 $cm^3$/100 g of body weight. After anesthesia, the intraoral surgical field was disinfected with a povidone–iodine topical antiseptic.

Three types of wound were randomly made using either a stainless-steel scalpel (Bard-Parker No. 15; Feather Safety Razor, Osaka, Japan), a diode laser (Green-10; LAMBDA, Vicentina, Italy) or an Er,Cr: YSGG laser (Waterlaser MD; BIOLASE, San Ramon, CA) in the oral buccal mucosa of each guinea pig. Each incision was 15 mm in length and was performed to the surface of the periosteum with no flap reflection. The distance between each incision was ≈15 mm. A stainless-steel scalpel was used per animal. All wounds were closed using 5-0 absorbable sutures (Vicryl; Ethicon, Somerville, NJ).

The diode laser was set at a wavelength of 810 nm, with 2 W of power, a pulse length of 0.5 ms continuous wave and an aiming beam of 635 nm (red spectrum). The fiber tip diameter was 300 μm and a non-contact, continuous mode was used. The Er,Cr:YSGG laser apparatus was set at a wavelength of 2.78 μm, power 3 W, 15% water, 18% air spray, pulse duration of 140 μs, repetition rate of 25 Hz, energy density of 300 $mJ/cm^2$ and spot size of 0.6 mm with a non-contact mode (the distance to the soft tissue was 0.5 mm to 1.0 cm). The lasers were calibrated by the manufacturer before the study. The same person who was familiar with laser applications performed all the surgical procedures under aseptic conditions. Antibiotics (terramycin 0.5 ml, intramuscular) were administered for 4 days following the surgical procedures.

### *Histological preparations and examinations*

Five guinea pigs were sacrificed on each of Days 1, 3, 5 and 7 post-surgery. Four guinea pigs were sacrificed at Day 14 post-surgery. Biopsy samples from each oral mucosa wound were fixed immediately in 10% neutral buffered formalin. After embedding in paraffin, 4-μm sections were prepared from across the middle of each wound, stained with hematoxylin–eosin and then examined by light microscopy. An inflammatory response was evaluated and scored using a scale ranging from 0 to 3 based on the degree of neutrophil, histiocyte and lymphocyte infiltration.

### *Immunostaining procedure and evaluation*

Microtome sections were evaluated by immunohistochemical staining for the detection of TNF-α and TGF-β1 using a specific assay system (Super sensitive polymer-HRP detection system, secondary antibody conjugated with horseradish peroxidase polymer kit, QD420-YIK; BioGenex, Irvine, CA). Microtome sections were deparaffinized using serial xylene and ethanol baths. After rehydration, slides were heated in a microwave oven with 0.01 M citrate buffer for 10 min, and were then treated with 3% $H_2O_2$ in methanol for 10 min at room temperature. Slides were washed two to three times with 0.1 M phosphate-buffered saline (PBS) at pH 7.4 for 5 min and were then incubated for 1 h with primary antibodies (TNF-α, mouse monoclonal; Hycult Biotechnology, Uden, The Netherlands; TGF-ß1, rabbit polyclonal; BioVision, Mountain View, CA) at room temperature. Slides were washed with PBS for 15 min and then incubated with an enhancer reagent (HK 518-50K; BioGenex) for 20 min at room temperature. After washing with PBS, slides were incubated with polymer–horseradish peroxidase (HK 519-50K; BioGenex) for 30 min. After a final wash in PBS, slides were treated with diaminobenzidine (Dako, Glostrup, Denmark) to obtain a dark brown staining of the immunoreaction. Sections were counterstained with Mayer's hematoxylin for 2 min, dehydrated in ethanol, cleared in xylene and mounted in Canada balsam. The expressions of TNF-α and TGF-β1 were evaluated as the percentage of positively stained cells and the immunostaining intensity by using a scale ranging from 0 to 3. After an observer determined that the scale for the sweat gland was 2 using the avidin–biotin–peroxidase complex method, the immunostaining intensity for each specimen was evaluated by comparison with the immunostaining intensity of the sweat gland. The sweat gland tissue served as a positive control for the immunohistochemistry, because the sweat gland tissues are always stained strongly positive independent of antigen existence. All slides were evaluated and scored by an independent observer using a light microscope (Axiophot, Zeiss, Germany). The observer was blinded as to the type of incision modality and the relative age of the wound.

## Results

### *Inflammatory response*

The inflammatory responses (IRs) for scalpel wounds (scale 2) and Er,Cr:YSGG laser wounds (scale 1.8) were greater than the IR for diode laser wounds (scale 1.4) at Day 1 post-surgery. However, at Day 3 post-surgery the IRs for scalpel wounds (scale 1.6) and Er, Cr:YSGG laser wounds (scale 1.8) appeared to be less than that for diode laser wounds (scale 2.2). At Day 5 post-surgery the number of inflammatory cells infiltrated had rapidly decreased in all treatment groups (Figures 1 and 2).

### *TNF-α expression*

The highest level of TNF-α expression was observed at Day 1 post-surgery (50%) for diode laser wounds

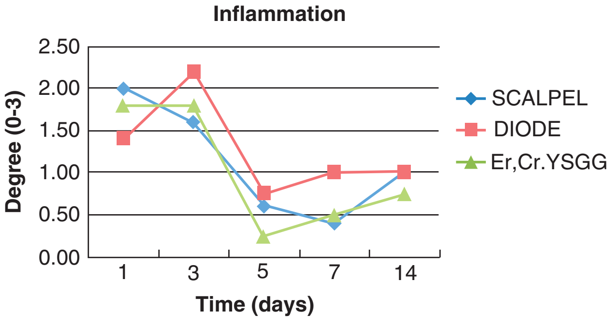

Figure 1. Results of histologic analysis of wounds produced by scalpel, diode laser and Er,Cr:YSGG laser.

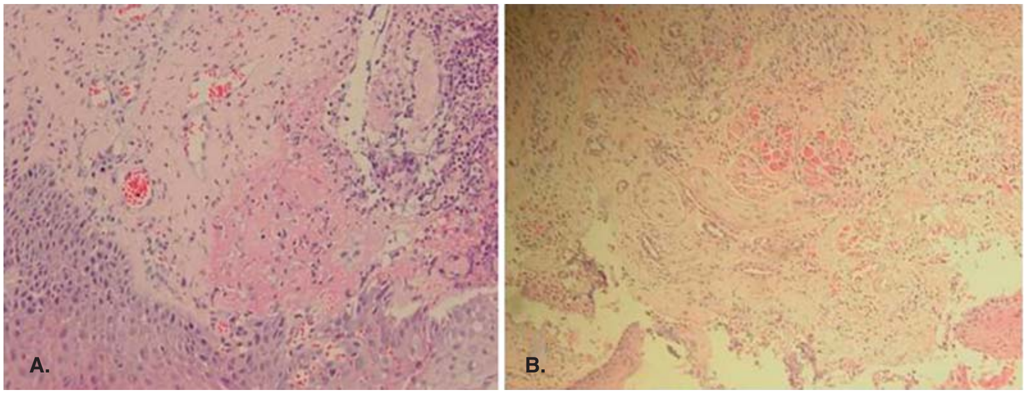

Figure 2. Histologic appearance of a diode laser wound at Day 3 post-surgery (A), showing more inflammatory cell infiltrate than observed in an Er,Cr:YSGG laser wound at Day 5 post-surgery (B). Original magnification ×100.

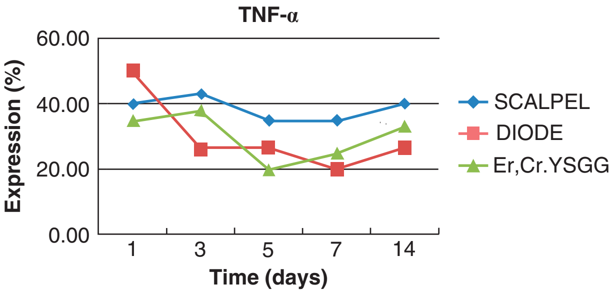

Figure 3. TNF-α expression for scalpel, diode laser and Er,Cr:YSGG laser wounds.

and at Day 3 post-surgery for scalpel wounds (43%) and Er,Cr:YSGG laser wounds (38%). The lowest level of TNF-α expression was found at Day 5 post-surgery for scalpel wounds (35%) and Er,Cr:YSGG laser wounds (20%) and at Day 7 post-surgery for diode laser wounds (20%). Overall, during the wound healing period, TNF-α expression was higher in scalpel wounds than in Er,Cr:YSGG laser wounds (Figures 3 and 4).

Immunostaining intensities were highest at Day 3 post-surgery and lowest at Day 7 post-surgery in all treatment groups (Figure 5). The highest intensity of TNF-α immunostaining was found primarily in neutrophils at Days 1 and 3 post-surgery and in fibroblasts at Day 14 post-surgery (Figure 6).

### *TGF-β1 expression*

For scalpel wounds, a lower level of TGF-β1 expression was seen until Day 3 post-surgery and a higher level from Day 7 post-surgery compared to laser wounds. The level of TGF-β1 expression was increased until Day 3 post-surgery for scalpel wounds and for diode laser wounds. After the expression level decreased up to Day 5 post-surgery, it then increased until Day 14 for both types of wound. The lowest level of TGF-β1 expression for Er,Cr:YSGG laser wounds was observed at Day 7 post-surgery (42%; Figure 7).

The intensity of TGF-β1 immunostaining decreased until Day 5 post-surgery, and then increased until Day 14 post-surgery in all treatment groups. The intensity of immunostaining was highest at Day 1 post-surgery for diode laser wounds (Figure 8).

## Discussion

Although scalpels have many advantages, including accuracy and minimal tissue injury, the resulting wound allows extravasation of blood and lymph fluid which, in turn, may result in edema, swelling and a prolonged IR [15,16]. The coagulation properties associated with the use of a diode laser are particularly beneficial during removal of vascular lesions. Romanos et al. [17] examined healing of oral soft tissue wounds following application of a 980-nm

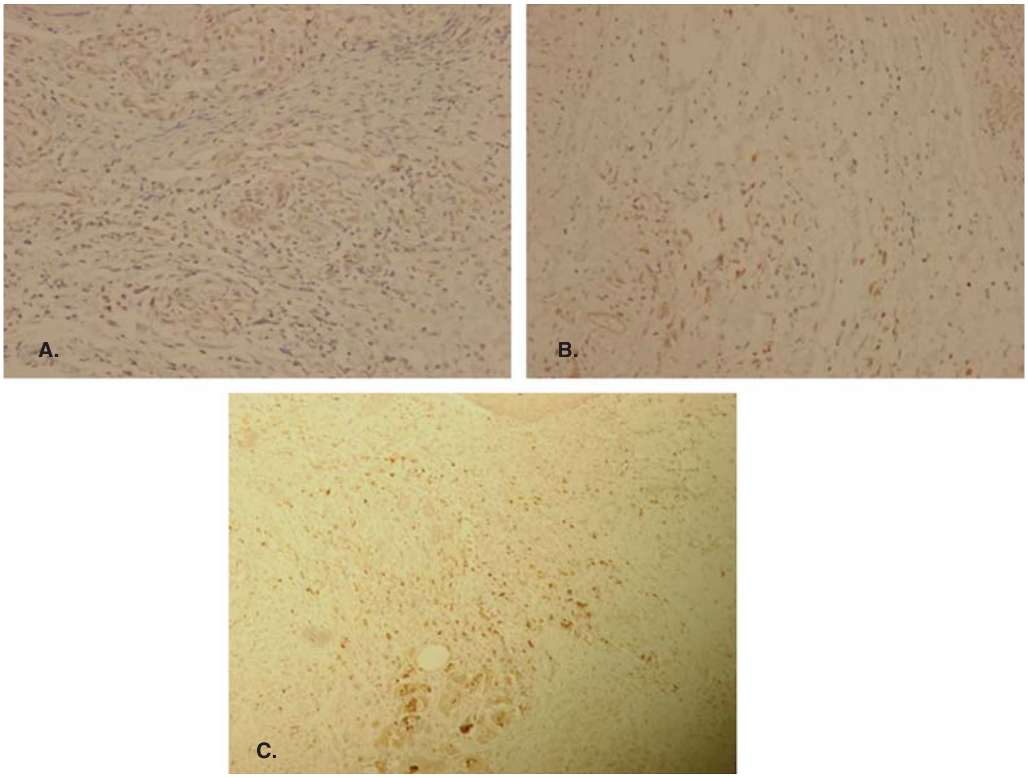

Figure 4. TNF-α expression was higher at day 1 post-surgery (A) than at day 3 (B) and at day 7 (C) post-surgery for diode laser wounds.

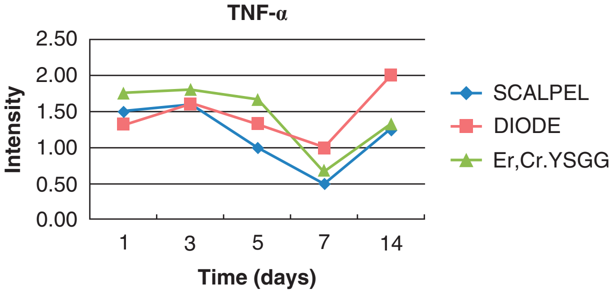

Figure 5. Analysis of the intensity of TNF-α immunostaining in wounds produced by scalpel, diode laser and Er,Cr:YSGG laser.

diode laser and reported that clinical findings included sufficient hemostasis, precise incision margins, lack of swelling, pain or scar formation, and good wound healing. In a similar manner, studies using the Er,Cr:YSGG laser have reported improved healing with a faster recovery time and less trauma to the target tissue when compared to traditional surgical modalities [18,19]. Luomanen [3] compared incisions made by scalpel and laser and reported a delay in capillary proliferation at laser-treated sites during the early healing phase. In addition, other studies have reported a more dense inflammatory cell infiltrate in laser wounds compared to scalpel-induced wounds [15,16]. In the present study, when compared to the diode laser wounds, the IR for both the scalpel and Er,Cr:YSGG laser wounds appeared greater at Day 1 but lower at Day 3 post-surgery. In all treatment groups the intensity of inflammatory cell infiltrate was significantly less at Day 5 post-surgery.

Bacterial lipopolysaccharide (endotoxin) is a stimulus for cellular cytokine production. Following activation by endotoxins, mononuclear inflammatory cells (i.e. macrophages and lymphocytes) are stimulated to produce proinflammatory cytokines, including TNF-α and interleukin-1. These proinflammatory molecules are responsible for a wide range of bioactivities of major importance to the pathogenesis of the IR. These effects may be acute (fever, shock, altered levels of hormones, coagulopathies, adherence of leukocytes to endothelium), subacute (tumor cell injury, muscle breakdown, development of humoral and cell-mediated immunity) or chronic (wound healing, fibrosis, erosion of cartilage, remodeling of bone) [20,21]. Hübner et al. [22] reported a strong, early induction of TNF-α expression after cutaneous injury. The highest level of this cytokine was seen as early as 12–24 h after wounding. During the early phase of wound repair, this cytokine was predominantly expressed in polymorphonuclear leukocytes. At the later stage of the repair process, expression of TNF-α was also seen in macrophages [22].

In the present study, the level of TNF-α expression was found to be higher for diode laser wounds than for scalpel wounds or Er,Cr:YSGG laser wounds at Day 1 post-surgery. This suggests that a diode laser may cause greater tissue damage than a scalpel or an Er, Cr:YSGG laser.

Almost all cells have TGF-β1 receptors, which gives a considerable spectrum to the activity of this growth factor. Its activities include context-specific inhibition or stimulation of cell proliferation, control of extracellular matrix (ECM) synthesis and degradation, control of mesenchymal–epithelial interactions during embryogenesis, mediation of cell and tissue responses to injury, control of carcinogenesis and modulation of immune functions. The general functions of TGF-β in tissue regeneration are chemotactic for inflammatory cells (primarily monocytes) and promotion of synthesis of the ECM (collagen, fibronectin, tenascin, etc.). TGF-β1 acts as a mediator in the three main phases of wound healing and also plays a role in the late phase of matrix formation, i.e. fibrosis [13]. Thus, TGF-β1 expression is seen primarily in inflammatory cells during the early wound healing period. However, increased TGF-β1

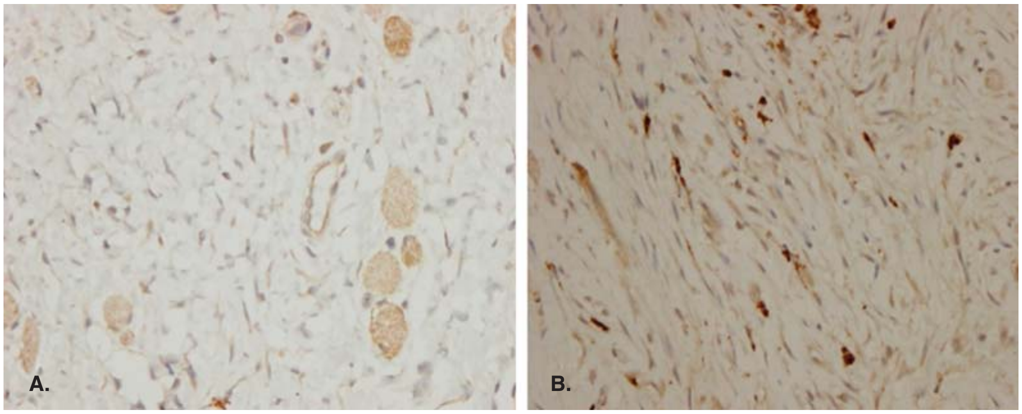

Figure 6. The intensity of TNF-α immunostaining in fibroblasts was higher at Day 14 post-surgery for diode laser wounds (B) than at Day 7 post-surgery for the Er,Cr:YSGG laser wounds (A). Original magnification ×400.

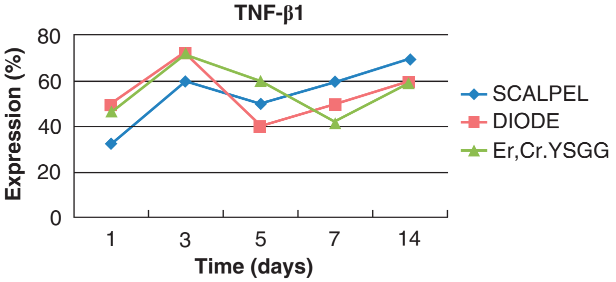

Figure 7. TGF-β1 expression for scalpel, diode laser and Er,Cr:YSGG laser wounds.

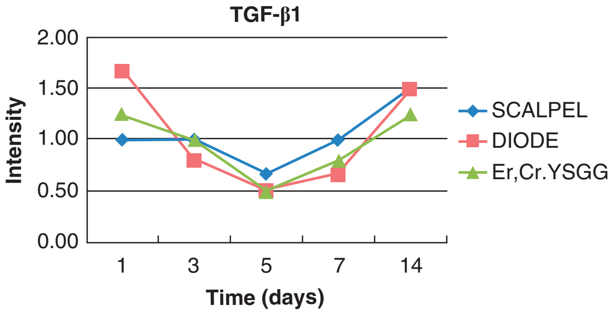

Figure 8. The intensity of TGF-β1 immunostaining for scalpel, diode laser and Er,Cr:YSGG laser wounds.

expression is also seen in fibroblasts during the late wound healing period.

In this study, a lower level of TGF-β1 expression was observed until Day 3 post-surgery for scalpel wounds compared to laser wounds. It can be considered that the damage resulting from using a scalpel was lower when compared to that after the laser surgical procedures for early wound healing. The intensity of immunostaining was highest at Day 1 post-surgery for diode laser wounds. This result indicates that cellular injury was higher for diode laser wounds than for Er,Cr:YSGG laser wounds.

This study reveals that the diode laser can be considered a good incisional device for oral mucosa incisions; however, more tissue damage resulted from its use compared to that using a scalpel or an Er,Cr:YSGG laser.

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