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ORIGINAL ARTICLE

A comparative histological and immunohistochemical study of wound healing following incision with a scalpel, CO_2 laser or Er,Cr:YSGG laser in the Guinea pig oral mucosa

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Abstract

Purpose. This study was undertaken to compare wound healing following incisions with either a scalpel, CO_2 laser or Er,Cr:YSGG laser in Guinea pig oral mucosa. ***Materials and methods.*** Three types of wounds were randomly introduced with either a stainless steel scalpel, CO_2 laser or Er,Cr:YSGG laser in the buccal mucosa of each of 22 Guinea pigs. Four Guinea pigs were sacrificed on day 1, day 3 and day 5 post-surgery. Five Guinea pigs were sacrificed on day 7 and day 14 post-surgery. Biopsy samples from each oral mucosa wound were examined and the expression of TNF-α and TGF-β1 was determined by immunohistochemical staining. ***Results.*** At day 3 post-surgery, the histological pattern of the healing process was similar in the scalpel and Er,Cr:YSGG laser wounds and there were more ulcerations present in the CO_2 laser wounds than in the scalpel and Er,Cr:YSGG laser wounds. The level of TNF-α expression was twice in the laser wounds that in the scalpel wounds. A higher level of TGF-β1 expression was seen at day 7 post-surgery and a lower level at day 14 post-surgery in the CO_2 laser wounds than in the scalpel and Er,Cr:YSGG laser wounds. ***Conclusions.*** The Er,Cr:YSGG laser has many advantages for oral surgery due to a low inflammatory response and minimal damage of the tissue. Although a CO_2 laser has better hemostatic ability, its use causes greater tissue damage than a scalpel and Er,Cr:YSGG laser. However, further larger studies would be needed before fully endorsing its widespread use.

Key Words: *scalpel, CO_2 laser, Er, Cr:YSGG laser, TNF-α, TGF-β1*

Introduction

Various devices including the laser, scalpel and electrosurgical devices have been introduced in recent years to achieve hemostasis with minimal tissue injury during surgery. After the ruby laser was discovered by Maiman in the 1960s, many medical applications utilizing the laser were developed, particularly for ophthalmological, dermatological and general surgery. Stern and Sognnaes in 1964 began to investigate the possible uses of the ruby laser in dentistry [1–3].

The scalpel has been used for many years because of its ease of use, accuracy and minimal damage to adjacent tissue. The need for hemostasis in highly vascular areas such as the head and neck region led to the widespread use of electrosurgery. Electrosurgery provides enhanced hemostasis by sealing blood vessels before cutting. However, reports have suggested that healing is delayed and wound strength is lower for electrosurgery wounds as compared to scalpel wounds [4].

With laser irradiation, the major advantages are the production of local hemostasis—creating a virtually bloodless surgical field—bacterial elimination and a contact-free incision. Post-operative pain is also notably minimized with the use of laser incisions [5,6].

The carbon dioxide (CO_2) laser has become one of the most useful devices in oral surgery because of the following advantages: a bloodless operating field, precise dissection and absence of retractility [7]. With the recent development of solid-state lasers, a new type of erbium laser, the Er,Cr:YSGG (erbium, chromium:yttrium, scandium, gallium, garnet) laser has been developed with an emission wavelength of 2.78 μm. Energy from this laser is absorbed effectively

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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 in both hard and soft tissues [3,8].

Although a number of ablative-laser techniques have been successfully developed and used in the clinical setting, the biomolecular processes influencing wound healing after exposure to laser energy are not well elucidated [9–11].

Tumor necrosis factor (TNF) has been shown to contribute to several events that are essential for the initiation of an inflammatory response and ultimately tissue destruction. TNF can induce up-regulation of adhesion molecules in leukocytes and endothelial cells and stimulate the production of chemokines, which represent critical elements in the recruitment of circulating leukocytes. TNF can also induce expression of other mediators that amplify or sustain the inflammatory response such as prostaglandins and TNF can stimulate the production of lytic enzymes such as collagenase and enhance bacterial killing and phagocytic activity. Furthermore, TNF is synergistic in its capacity to stimulate bone resorption [12–14]. Transforming growth factor-β (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 [15].

This study was undertaken to compare the histology and immunohistochemistry of wound healing following incision with a scalpel, CO_2 laser or Er,Cr:YSGG laser in the Guinea pig oral mucosa. The immunohistochemical analysis used polyclonal antibodies that were specific for TNF-α and TGF-β1.

Materials and methods

The Committee on the Use and Care of Animals and the Institutional Review Board (IRB) of the Catholic University of Korea approved the study.

Surgical procedures

Twenty-two male Guinea pigs that weighed 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 anesthetized with a mixture (1:8) of xylazine hydrochloride (Rompun, Bayer in Korea, Seoul, Republic of Korea) and ketamine (Ketatar, Yuhan, Seoul, Republic of Korea) that was administered slowly by intraperitoneal injection as a dose of 0.5 cc/100 g of body weight. After anesthesia, the intra-oral surgical field was sterilized with Betadine solution. Three types of wounds were randomly introduced with the use of a stainless steel scalpel (Bard-Parker No. 15, Feather Safety Razor, Osaka, Japan), CO_2 laser (Leaders Plus, DoctorMed, Seoul, Republic of Korea) or Er,Cr:YSGG laser (Waterlaser MD, BIOLASE, San Ramon, CA) in the oral buccal mucosa of each Guinea pig (Figure 1). Each incision that measured 15 mm in length was performed. The tissues were incised without any elevation of a full mucoperiosteal flap. All wounds were closed with the use of 5-0 absorbable sutures (Vicryl, Ethicon, Somerville, NJ).

The CO_2 laser apparatus was set at a wavelength of 10 600 nm, power 4 W in CW, pulse duration 400 μs, interval 5 ms and spot size 0.2 mm. 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 140 μs and repetition rate of 25 Hz.

The lasers were calibrated by the manufacturer before the study. The same operator under aseptic conditions performed all of the surgical procedures. Antibiotics (terramycin 0.5 mL, IM) were administered for 4 days following the surgical procedures.

Histological preparation and examinations

Four Guinea pigs were sacrificed each at day 1, day 3 and day 5 post-surgery. Five Guinea pigs were

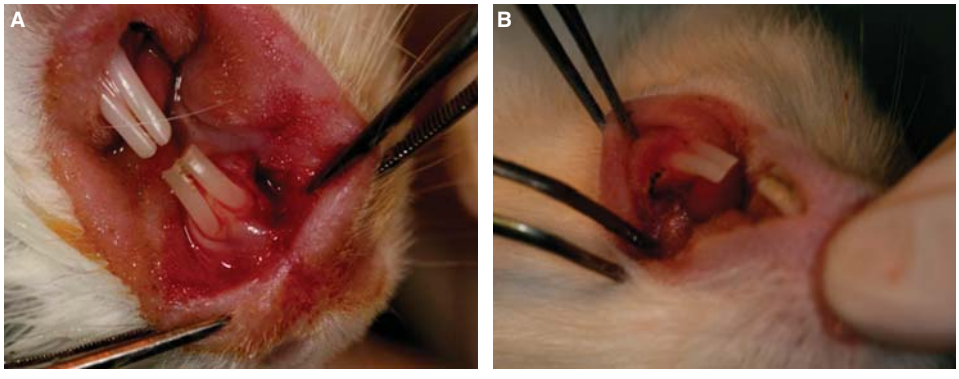

Figure 1. Er,Cr:YSGG laser (A) and CO_2 laser wounds (B). There appeared to be less intra-surgical bleeding in the CO_2 laser wound.

sacrificed at day 7 and day 14 post-surgery. Biopsy samples from each oral mucosa wound were fixed immediately in 10% neutral buffered formalin. After paraffin wax-embedding, sections (4 μm) were prepared across the middle of each wound and were stained with hematoxylin and eosin and were then examined by light microscopy. The inflammatory responses based on the degree of neutrophil, histiocyte and lymphocyte infiltration, ulceration, fibrosis and granulation tissue were evaluated and scored using a scale of 0–3.

Immunostaining procedure and evaluation

Microtome sections were evaluated by immunohistochemical staining for the detection of TNF-α and TGF-*β*1 by the use of 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 (4 μm thick) 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 with 0.1 M phosphate-buffered saline (PBS) at pH 7.4 2–3 times for 5 min and were then incubated for 1 h with primary antibodies (TNF-α, mouse monoclonal, Hycult Biotechnology, Uden, The Netherlands; TGF-*β*1, rabbit polyclonal, BioVision, Mountain View, CA) at room temperature. All of the treated slides were washed with PBS for 15 min and were then incubated with an enhancer reagent (HK 518-50K, BioGenex) for 20 min at room temperature. After re-washing with PBS, slides were incubated with the polymer-HRP (HK 519-50K, BioGenex) for 30 min. After a final wash in PBS, slides were treated with di-aminobenzidine (DAKO, Glostrup, Denmark) to obtain 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 by the percentage of the positive-stained cells and the immunostaining intensity by using a scale of 0–3. After the observer determined the scale of the sweat gland as 2, the immunostaining intensity of each specimen was evaluated by a comparison with the the immunostaining intensity of the sweat gland. All slides were evaluated and scored by an independent observer with the use of a light microscope (Axiophot, Zeiss, Germany) who was blinded as to the type of incisional modality used and the relative age of the wound.

Results

Inflammatory response (IR)

Although the IR in the laser wounds (scale 2) appeared to be greater than that in the scalpel wounds (scale 1.75) at day 3 post-surgery, the IR in the laser wounds (scale 0.75) appeared to be smaller than that of the scalpel wounds (scale 1.0) at day 5 post-surgery. For the laser wounds, the inflammatory cells infiltrated more in the wounds at day 3 than at day 1 post-surgery. The least inflammatory cells infiltrated at day 5 post-surgery for all three groups of animals (Figures 2 and 3A).

Ulceration

There were more ulcerations in the CO_2 laser wounds (scale 1.67) than in the scalpel wounds and Er,Cr:YSGG laser wounds (scale 1.25) at day 3 post-surgery. No ulcerations were observed in the laser wounds after day 5 post-surgery (Figure 3B).

Fibrosis

More fibrosis were present in the scalpel wounds (scale 2.25, 2.6) and Er,Cr:YSGG laser wounds (scale 2.0, 2.4) than in the CO_2 laser wounds (scale 1.5,1.4) at day 3 and day 14 post-surgery (Figure 3C).

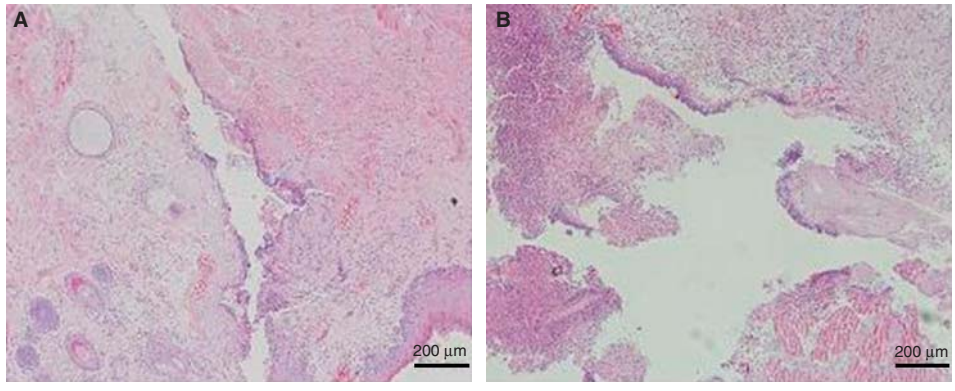

Figure 2. Histological appearance in the CO_2 laser wound showed more infiltration of inflammatory cells in the wound at day 3 post-surgery (B) than day 1 post-surgery (A).

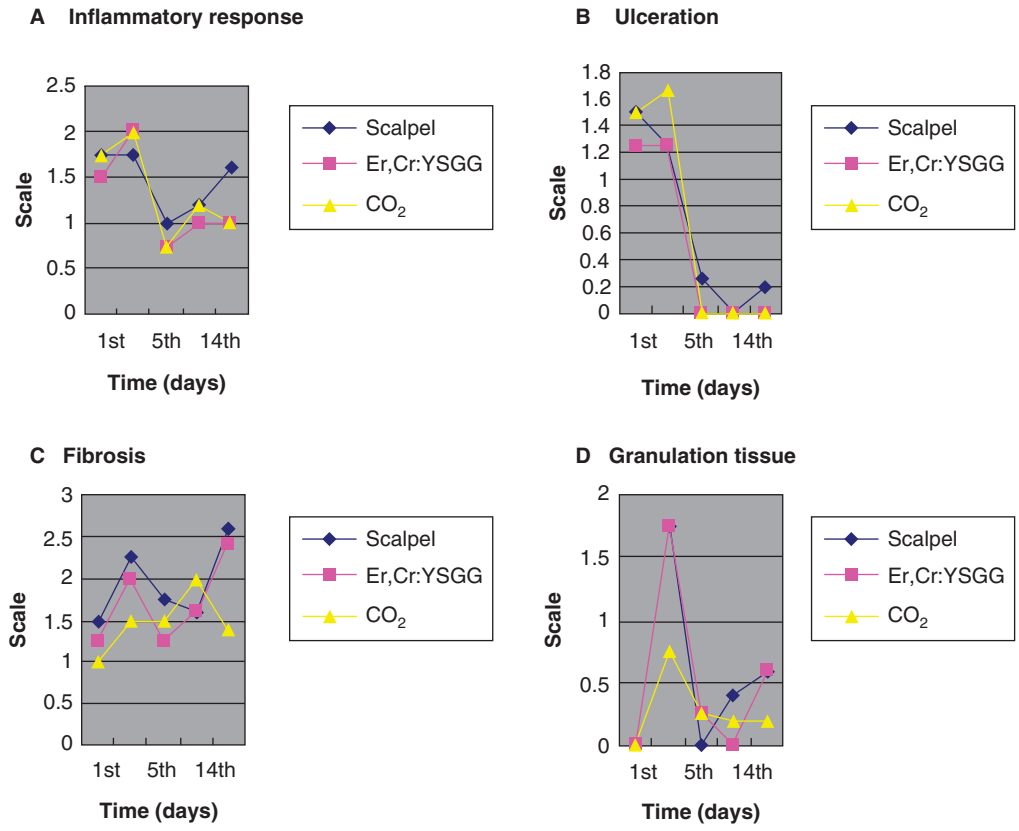

Figure 3. Histological analysis of the scalpel, CO_2 laser and Er,Cr:YSGG laser wounds.

Granulation tissue

More granulation tissue were found in the scalpel wounds and Er,Cr:YSGG laser wounds (scale 1.75, 0.6) than in the CO_2 laser wounds (scale 0.75, 0.2) at day 3 and day 14 post-surgery. The granulation tissue reached its maximum after 3 days for all three groups of animals (Figure 3D).

TNF-α expression

TNF-α expression was found mainly in the multinuclear macrophages and the neutrophils of the connective tissue (Figure 4). The highest level of TNF-α expression was found at day 1 post-surgery (40%) in the scalpel wounds and at day 3 post-surgery (45%) in the laser wounds. Twice the level of TNF-α expression was seen in the laser wounds (45%) than in the scalpel wounds (27.5%) at day 3 post-surgery. TNF-α expression was higher in the CO_2 laser wounds as compared with the Er,Cr:YSGG laser wounds during the wound healing period. The intensity of immunostaining was the highest at day 3 post-surgery for all three groups of animals (Figures 5A and B).

TGF-β1 expression

TGF-β1 expression was seen mainly in the inflammatory cells during the early wound healing period. However, increased TGF-β1 expression was seen in fibroblasts during the late wound healing time (Figure 6). The highest level of TGF-β1 expression was found at day 3 post-surgery in the scalpel wounds (82.5%) and in the Er,Cr:YSGG laser wounds (65%). After the expression level decreased until day 7 post-surgery, the level increased further until day 14 in both types of wounds. A high level of TGF-β1 expression was seen at day 3 post-surgery (65%) and at day 7 post-surgery (66%) in the CO_2 laser wounds. In the CO_2 laser wounds, a higher level of TGF-β1 expression was seen at day 7 post-surgery and a lower level at day 14 post-surgery than for the scalpel and Er,Cr:YSGG laser wounds. Although the level of TGF-β 1 expression was always higher in the scalpel wounds than in the Er,Cr:YSGG laser wounds during the healing process, the pattern of the level change in both wounds was similar. The intensity of immunostaining was the highest at day 3 post-surgery for all three groups of animals (Figures 5C and D).

Discussion

Advantages of the use of lasers include greater precision, a relatively bloodless surgical and post-surgical course, sterilization of the surgical area, minimal swelling and scarring, coagulation, vaporization and cutting, minimal or no suturing and much less or no post-surgical pain [16–18].

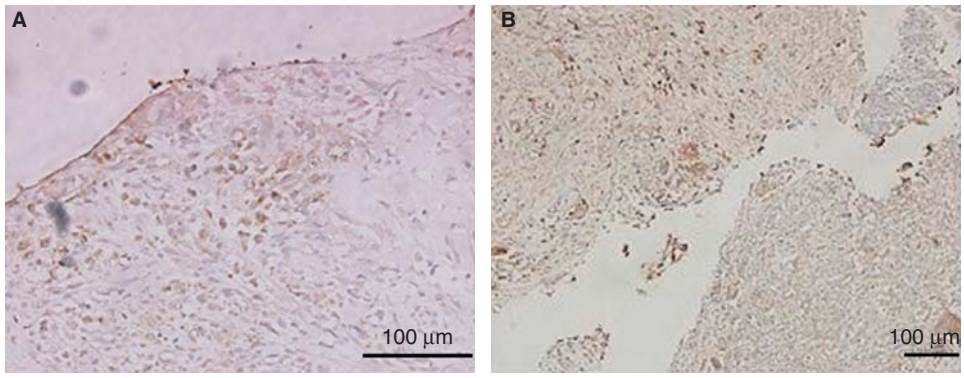

Figure 4. TNF-α expression in Er,Cr:YSGG laser (A) and scalpel (B) wounds. Positivity is seen in multinuclear macrophages at day 3 post-surgery (A) and in neutrophils at day 5 post-surgery (B) of the connective tissue.

Although biostimulatory effects of laser irradiation on cell proliferation and wound healing have been reported, the biomolecular processes influencing wound healing after exposure to laser energy have not been well presented [10].

As the CO_2 laser beam has a wavelength of 10.6 μm and is absorbed by water, resulting in vaporization of the intra- and extra-cellular fluid and disintegration of the cells, the CO_2 laser offers an alternative means to eradicate oral mucosal lesions [1,19–21]. Although the CO_2 laser is a very useful device in oral surgery, the main disadvantage of the use of the CO_2 laser is the thermal side-effects produced [22]. Better results are obtained with the use of Nd:YAG and Er:YAG lasers because of the physical characteristics [23]. In particular, the Er:YAG laser operates at a wavelength

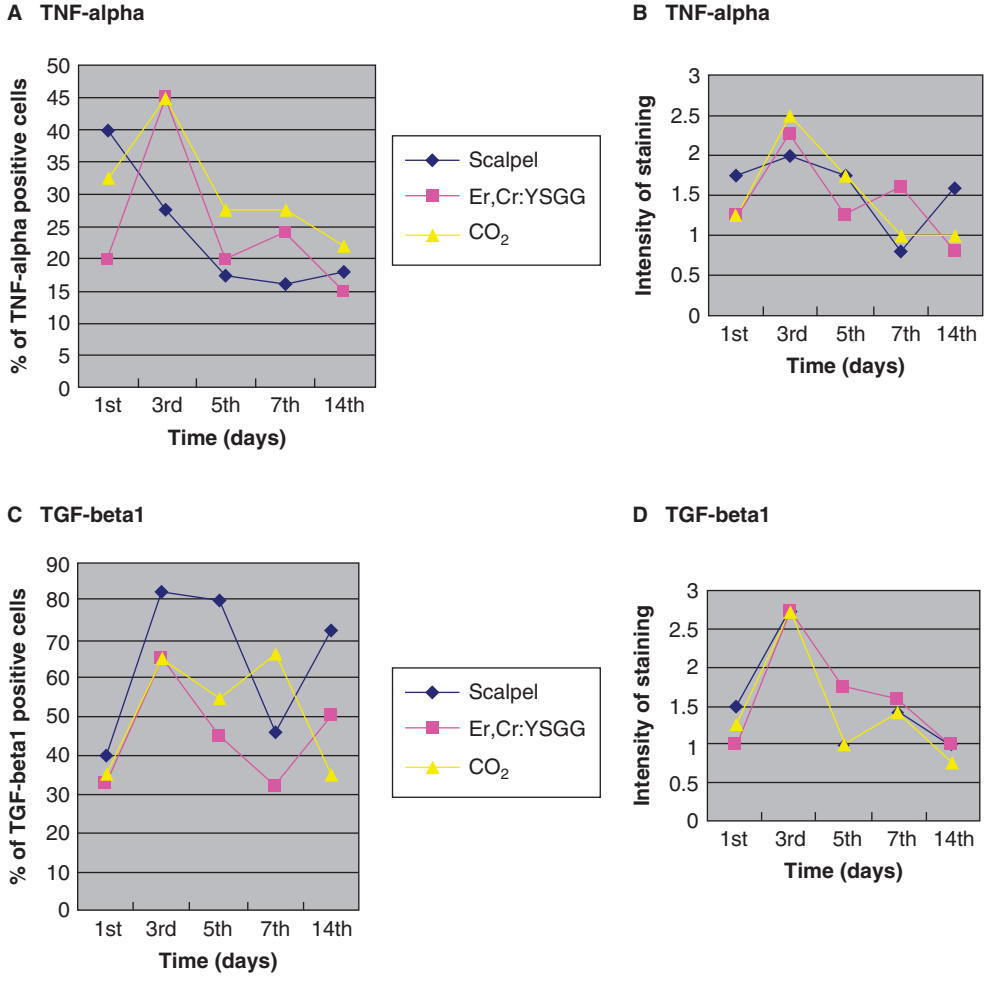

Figure 5. TGF-β1 expression in Er,Cr:YSGG laser (A) and scalpel (B) wounds. Detection of TGF-β1 expression is seen in multiple inflammatory cells at day 3 post-surgery (A) and in fibroblasts at day 7 post-surgery (B).

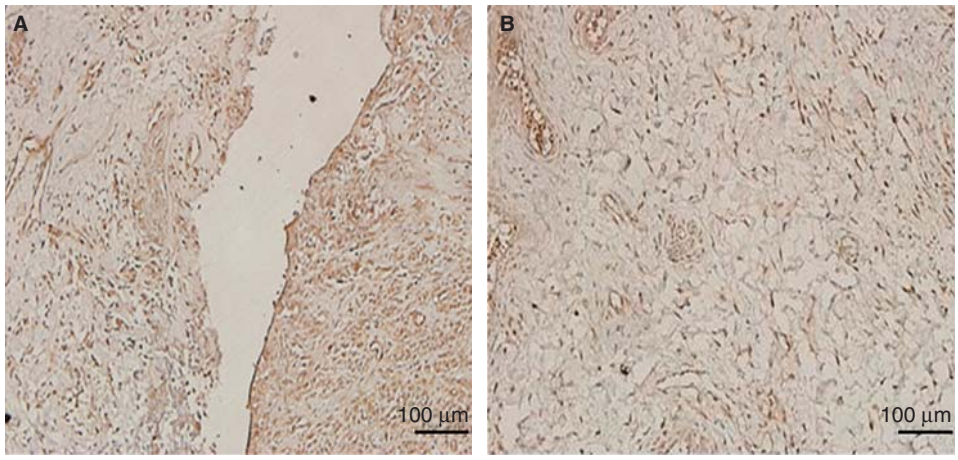

Figure 6. TNF-α and TGF-β1 expression in the scalpel, Er,Cr:YSGG laser and CO_2 laser wounds.

of 2.94 μm, which is well absorbed by water. The water absorption coefficient of the Er:YAG laser is greater than that of CO_2 and Nd:YAG lasers, therefore the Er:YAG laser seems to be particularly useful in soft tissue surgery when no coagulation effect is desired [7].

Liboon et al. [4] compared the scalpel, CO_2 laser, electrosurgery unit and constant-voltage electrosurgery unit in an effort to assess their value in a swine model. Histological damage, as expected, was minimal with a scalpel. The extent of epithelial damage lateral to the wound edge and the extent of collagen denaturation were minimized with use of a scalpel. The wounds created by all four instruments displayed intact epithelium by 4 weeks and granulation tissue peaked at 4 weeks with the use of the scalpel and CO_2 laser [4]. Sinha and Gallagher [5] compared tissue healing histopathologically when a steel scalpel, ultrasonic scalpel, monopolar or bipolar electrosurgical instruments or CO_2 laser was used in an animal oral surgery model. Tissue re-epithelialization was fastest with the use of the steel scalpel and ultrasonic scalpel (complete by day 7 post-surgery). Complete re-epithelialization of the wounds of all treatment groups occurred by day 28 post-surgery [5]. Rizoiu et al. [3] compared healing microscopically with the Er,Cr:YSGG laser and conventional scalpel in New Zealand white rabbits. Laser wounds showed minimal to no hemorrhage and re-epithelialization and collagenization were found to occur by day 7 post-surgery with the use of both a laser and scalpel. Zaffe et al. [7] investigated the morphological, histochemical and immunocytochemical changes after the use of CO_2 or Er:YAG laser irradiation in 40 oral mucosa biopsies of young patients and suggested that the Er:YAG laser may be routinely used in surgery as its use causes minimal damage to the epithelial tissue, a low inflammatory reaction, a quicker healing process and a lower risk of scarring.

In the present study, the pattern of the healing process was similar histologically for the scalpel wounds and Er,Cr:YSGG laser wounds and there were more ulcerations in the CO_2 laser wounds than in the scalpel wounds and Er,Cr:YSGG laser wounds at day 3 post-surgery.

The classic stimulus for cellular cytokine production is bacterial lipopolysaccharide (endotoxin). Upon activation by bacterial endotoxins, mononuclear inflammatory cells, such as macrophages and lymphocytes, start to produce TNF-α. After intracellular synthesis, TNF-α is transported to the cell surface and solubilized by a TNF-α converting enzyme (TACE), thus becoming able to affect its target cells auto- and paracellularly [24,25].

In the present study, the level of TNF-α expression was twice as high in the laser wounds than in the scalpel wounds at day 3 post-surgery. It can be considered that damage from the use of a scalpel was lower when compared to damage after the laser surgical procedures for early wound healing. TNF-α expression was higher in the CO_2 laser wounds as compared with the Er,Cr:YSGG laser wounds during the wound healing period. This result indicates that cellular injuries were also present in the Er,Cr:YSGG laser wounds, but to a very reduced extent.

TGF-β is the prototype of a multifunctional cytokine and appears in five isoforms, TGF-β1 to TGF-β5, but only the forms β1 to β3 are found in mammalian tissues. The general functions of TGF-β in tissue regeneration are chemotactic activity for inflammatory cells (mainly monocytes) and promotion of synthesis of the extracellular matrix (including collagen, fibronectin and tenascin). TGF-β1 is present in the three main phases of wound healing as a mediator and plays a role in the late phase of matrix formation by stimulation of fibrosis [26,27].

The results obtained in this study show that a higher level of TGF-β1 expression was found at day 7 post-surgery and a lower level of TGF-β1 expression

was found at day 14 post-surgery in the CO_2 laser wounds than in the scalpel wounds and Er,Cr:YSGG laser wounds. It is suggested that the inflammatory period was longer and it took more time to reach the phase of matrix formation in the CO_2 laser wounds than in the scalpel wounds and Er,Cr:YSGG laser wounds.

Our study supports the laser has many advantages for use in oral surgery, particularly the Er,Cr:YSGG laser, due to a low inflammatory response and less ulceration and minimal damage of the mucoperiosteal tissue. Although the CO_2 laser has better hemostatic ability, more tissue damage occurs than with the use of a scalpel and Er,Cr:YSGG laser. However, further larger studies would be needed before fully endorsing its widespread use.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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