Photomedicine and Laser Surgery
Volume 32, Number 1, 2014

Pp. 10–16
DOI: 10.1089/pho.2013.3509

Oral Soft Tissue Wound Healing After Laser Surgery With or Without a Pool of Amino Acids and Sodium Hyaluronate: A Randomized Clinical Study

Umberto Romeo, DDS, Fabrizio Libotte, DDS, Gaspare Palaia, DDS, PhD, Alexandros Galanakis, DDS, PhD, Gianfranco Gaimari, DDS, PhD, Gianluca Tenore, DDS, PhD, Alessandro Del Vecchio, DDS, and Antonella Polimeni, MDS

Abstract

Objective: The purpose of this study was to compare secondary intention healing of oral soft tissues after laser surgery with and without the use of a compound containing amino acids and sodium hyaluronate. ***Background data:*** Sodium hyaluronate has been successfully used in medicine to promote healing. It has not been studied in the healing of laser-produced wounds. ***Materials and methods:*** Excisional biopsy was performed in oral soft tissues with a potassium-titanyl-phosphate (KTP) laser (532 nm, SmartLite, DEKA, Florence, Italy) in 49 patients divided into two groups. In the study group (SG), 31 patients received a compound gel containing four amino acids and sodium hyaluronate (Aminogam®, Errekappa, Italy) after laser surgery; in the control group (CG), 18 subjects received no treatment involving a drug or gel. Numeric rating scale (NRS) was used to evaluate pain experienced after surgery [pain index (PI)]. Using a grid as a benchmark and computer software, the lesion area was measured after surgery (T_0) and after 7 days (T_1). A percentage healing index (PHI) was calculated indicating healing extension in 7 days. ***Results:*** SG cases showed an average PHI of 64.38 ± 26.50, whereas the average PHI in the CG was $47.88\% \pm 27.84$. Mean PI was 2.67 ± 0.96 for SG and 2.75 ± 0.86 for CG. A statistically significant difference was detected between the groups for PHI ($p = 0.0447$), whereas no difference was detectable for PI ($p = 0.77$). ***Conclusions:*** The use of a gel containing amino acids and sodium hyaluronate can promote faster healing via secondary intention in laser-induced wounds, although it does not seem to affect pain perception.

Introduction

In the past few years, lasers have brought many advantages to surgical procedures in dentistry, especially with the advent of different laser devices. They can be classified according to wavelength, active medium, power level, or biological effects generated.[1] Lasers bring more advantages to daily surgical procedures than does the scalpel, including a high degree of decontamination of the surgical area and minimal postoperative bleeding, particularly with the potassium-titanyl-phosphate (KTP) laser, the diode laser, and the Nd:YAG laser, as well as significantly decreased postoperative pain and inflammation.[1,2]

One procedure that leverages such lasers is oral biopsy. Biopsy is a surgical procedure performed to reach a clear diagnosis of a suspicious lesion. Two different types of biopsies exist: the incisional biopsy performed by taking one or more parts of a lesion, and the excisional biopsy performed by the excision of the whole lesion.[3]

One of the best advantages of the laser is wound healing by secondary intention, because of the intrinsic coagulation capacity, which makes this instrument a great help in daily surgical practice.[4] The absence of suture brings several advantages, including less stress, less discomfort for the patient, and less plaque accumulation around the operative site, the last being a condition that can delay normal tissue healing.[5]

It is, therefore, essential to reduce postoperative pain and, consequently, decrease the healing time of oral cavity soft tissues that are subjected to continuous trauma caused by mastication and phonation. Much scientific evidence has shown that wound healing can be facilitated and accelerated by the exogenous administration of hyaluronic acid (HA).

In 1985, West et al. demonstrated that the degradation products of HA could induce angiogenesis.[6] The angiogenic response was confirmed in 1994 and in 1997, and was later attributed to an intracellular effect on signaling pathways.[7–11]

Department of Oral Sciences, "Sapienza" University of Rome, Rome, Italy.

Furthermore, hyaluronic acid has been shown to contribute to the regulation of locomotion in ras-transformed cells.[12] The application of long-chain HA to gingiva resulted in decreased fibroblast proliferation, a finding shown *in vitro* with cultured fibroblasts.[13]

Evidence also exists regarding enhanced extracellular matrix remodeling following the application of HA matrices, as well as more ordered collagen deposition with less degradation.[14–17]

West et al.[18] demonstrated that the application of hyaluronidase (and, by extension, increased levels of HA fragments) causes increased scarring, whereas persistently raised levels of the macromolecule decrease fibroblast contraction.[19]

Therefore, there is evidence from scientific studies to indicate that HA might affect, predominantly in a beneficial manner, several of the components of wound healing.

For example, HA has been shown to enhance wound healing by stimulating fibroblasts to proliferate, increasing also the production of growth factors and the biosynthesis of several types of collagen.[20] With this in mind, HA has been used *in vivo* for a number of applications, resulting in some qualified success.

Laser biopsies produce wounds that heal via secondary intention and that usually have longer recovery times. The aim of this study was to understand if a compound consisting of amino acids and HA could speed up the wound healing process. Moreover, the study evaluated if the compound could modify postoperative pain perception.

Materials and Methods

Excisional biopsy was performed on a group of 49 nonsmoking patients between 27 and 70 years of age (mean age 45.5), not affected by systemic diseases, without problems of hemostasis and coagulation, and with good oral hygiene. Patients were referred to the Complex Operative Unit of Stomatology of the Department of Oral and Maxillo-Facial Sciences. All patients participating in the study were properly informed of its features and, according to the Declaration of Helsinki, had to read and sign a written informed consent before being enrolled for the clinical section. The study protocol was submitted for approval to the Ethics Committee of the "Sapienza" University of Rome (protocol reference: 2595/12).

Two population samples were taken into consideration for the study design. The selected patients were allocated randomly to one of the groups, using a tailored spreadsheet. An unbalanced randomization with a 3:2 ratio between the experimental and the control group was chosen, in order to acquire more information on the profile of the tested compound. The samples or groups were constituted as follows.

Study Group (SG): 31 patients
Control Group (CG): 18 patients

Patients did not show any oral mucosal diseases except for those requiring oral biopsy. Surgery involved clinically benign lesions (e.g., oral fibroma) in different areas of the oral cavity, such as the buccal mucosa, tongue, and lips. Local anesthesia with 1.8 mL of mepivacaine solution (Mepivacaina Pierrel, 30 mg/mL, injection solution 1.8 mL, Pierrel Spa, Milan, Italy) was administered before the beginning of each surgical intervention. After surgery, the excised lesions were sent to a pathologist for histological evaluation and diagnosis.

These lesions were excised with KTP laser (SmartLite, DEKA, Florence, Italy, 532 nm), with power settings of 1.5 W in continuous mode, fluence of 2123 J/cm^2, and optical fiber diameter of 300 μm. Power output was controlled before starting the excision with a portable power meter (Nova, Ophir-Spiricon, North Logan, UT). No sutures were applied to obtain wound healing via secondary intention, nor was any dressing applied to the wound. Patients were instructed to treat the wounded area with an antiseptic 0.2% chlorhexidine spray (Corsodyl, GlaxoSmithKline Consumer Healthcare, Milan, Italy) three times per day for 1 week.

Furthermore, a compound containing four amino acids (1% glycine, 0.15% L-leucine, 0.75% L-proline, and 0.10% L-lysine) and sodium hyaluronate (1.33%) [82.27% water, 14.4% excipients and preservatives: methyl parahydroxybenzoate, propyl parahydroxybenzoate, propylene glycol, tetrasodium ethylenediaminetetraacetic acid (EDTA)] (Aminogam, Errekappa Euroterapici, Milan, Italy) was randomly administered to the SG patients ($n = 31$; 16 males, 15 females), whereas patients in the CG ($n = 18$; 9 males, 9 females) received no further treatment. No other medications were prescribed (e.g., antibiotics). This compound (a quantity of 0.5 mL was dispensed from a sterile siringe) was applied on the postsurgical wound to promote healing and reduce pain, immediately after antiseptic therapy, three times per day for 1 week, with recommendations to massage the area for 1 min to help with absorption. The product was applied after routine meals and after regular oral care procedures at home. Furthermore, patients were given instruction not to take any liquid or solid foods and to abstain from rinsing their mouths for at least 30 min after the application of the compound, for more efficient distribution and absorption.

To evaluate wound healing, photographic controls were taken immediately after surgery (T_0) and 7 days after surgery (T_1). High resolution pictures were taken with a digital camera (Nikon D200, Nikon Corporation, Tokyo, Japan) using an L-shaped graph paper benchmark (with 1 mm intervals), placed on the sides of the lesion. The obtained high resolution pictures were edited using graphics software (Adobe Photoshop CS5, Adobe Systems, San Jose, CA) (Fig. 1). After delimitation of the lesion edges and tracing of a

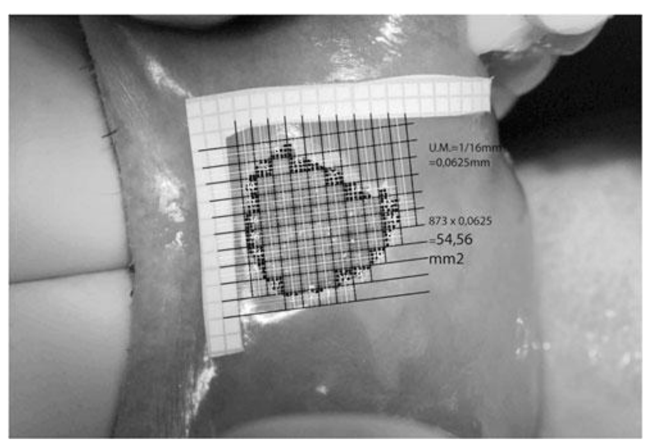

FIG. 1. Measurement of the lesion on a picture; the grid was traced using the paper benchmark placed next to the wound.

grid, the area was easily calculated in square millimeters to obtain two different values: the wound area at T_0 and the wound area at T_1. The resulting values were processed to obtain a percentage value, called percentage healing index (PHI). First, the T_1 area was divided by the T_0 area before it was multiplied by 100. The value thus obtained, the percentage unhealed index (PUI), represented the percentage of the lesion that did not heal over a 7 day period. The complementary value to this percentage was defined as the PHI (e.g., $PUI = T_1/T_0 \times 100 = 13.4\,mm^2/47.2\,mm^2 \times 100 = 28.39\%$; $PHI = 100 - PUI = 100 - 28.39 = 71.61\%$). Every patient underwent a postoperative discomfort evaluation 7 days after surgery (T_1) using the numeric rating scale (NRS) method, which recorded the greatest postsurgical pain experience using a number between 1 and 10. This score was named the "pain index" (PI).

During data analysis, lesions were divided into small size and large size, considering as the limit a hypothetical round wound of 5 mm of diameter, which has an area of $19.625\,mm^2$; therefore, wounds smaller than this area at T_0 were classified as small, whereas lesion larger than this area at T_0 were deemed as large. Wound areas in T_0 were tested with an unpaired parametric t test to understand if there were any major differences between the baseline areas of SG and CG.

Statistical analysis

Statistical analysis was performed using statistical software (GraphPad Prism 6.01 Trial, GraphPad Software, La Jolla, CA). For both groups and indexes, male and female, large and small lesions, mean and standard deviation (SD) values were obtained. PHI scores obtained in the two groups were tested with a D'Agostino–Pearson omnibus normality test to understand the feasibility of using a parametric test (i.e., assuming a Gaussian distribution of the samples).

An unpaired parametric t test was selected to detect significant differences between the two groups. An automatically performed F test confirmed that the groups were sampled from a population with equal variances. The same testing was performed for the PI values of the two groups. Statistical analysis was repeated separately for small and large wounds at T_0, and in order to detect differences between males and females for PHI and PI in both SG and CG a one way ANOVA test was applied, with a Tukey's multiple comparison post-test. When no statistically significant difference was found between the averages of two groups, a power testing was performed with an appropriate software (GraphPad StatMate 2, GraphPad Software, La Jolla, CA).

Results

All wounds healed without further complications within 3 weeks after surgery.

The t testing of the wound areas in SG and CG showed there was no statistically significant difference ($p = 0.31$) between the average baseline areas (T_0) in the two groups. Mean area value of SG was $29.42\,mm^2$ (SD = 20.54), whereas the mean area value of CG was $36.52\,mm^2$ (SD = 27.92).

The T_0 and T_1 wound areas, PHI values at T_0 and T_1, ages, and the PI scores are summarized in Table 1, along with gender of the patients and anatomical area of the wounds. SG showed an average PHI of 64.38% (SD = 26.50), whereas CG showed an average PHI of 47.88% (SD = 27.84), for a difference of 16.5% between the means of the two groups. Testing of the differences between the two groups with the unpaired t test revealed a significant difference ($p = 0.0447$) for PHI.

Mean age in SG was 46.10 (SD = 2.30), whereas in CG it was 44.44 (SD = 2.94).

The mean PI in SG was 2.67, with a SD of 0.96. The mean PI in CG was 2.75, with a SD of 0.86.

Statistical analysis did not find a significant difference between the two considered groups for PI ($p = 0.77$). Mean PI was not statistically different when considering large and small wounds in CG and in SG, nor when considering males and females in both SG and CG.

When considering only the wounds that in T_0 had an area of $<19.625\,mm^2$ (which is the equivalent area of a 5 mm diameter wound), the mean PHI for SG ($n = 11$) was 71.28% (SD = 26.05), whereas mean PHI for CG ($n = 7$) was 40.81 (SD = 19.24), for a difference of 31.1% between the means of the two groups. Testing of the differences between the two groups with the unpaired t test revealed a significant difference ($p = 0.022$) for PHI.

On the other hand, when considering only the wounds with an area in $T_0 > 19.625$ mm, mean PHI for SG ($n = 20$) was 61.47% (SD = 27.16), whereas mean PHI for CG ($n = 11$) was 51.74 (SD = 32.84), for a difference of 9.73% between the means of the two groups. Testing of the differences between the two groups with the unpaired t test did not reveal a significant difference ($p = 0.382$) for PHI.

Finally, the ANOVA testing among four groups, males and females in both SG and CG, did not reveal any statistically difference, as p value was > 0.05 ($p = 0.898$). In the Tukey's post-test the only statistically significant difference was between males subjects in SG and CG ($p < 0.05$).

Discussion

Since their introduction into dentistry, lasers have conferred numerous advantages from a clinical and surgical perspective.[21–27] Clinical experience gathered over the past few decades has suggested several advantages associated with use of laser rather than the scalpel during soft tissue surgery.[28]

In particular, the advantages of using KTP lasers in oral soft tissue surgery are its high cutting capacity, bloodless operative field, rapidity of use, and in some cases, reduced use of infiltrative anesthesia.[29]

Furthermore, an improvement in the repair processes and less postoperative pain can considerably improve the patient's postoperative comfort. In fact, HA can promote wound healing, thereby influencing the tissue repair process. HA plays an important role in wound healing as part of cell proliferation and migration. First, it furnishes a temporary structure in the early stages of the wound,[30] which promotes both the diffusion of nutritional supplies and the elimination of waste products from cell metabolism. Furthermore, HA is intimately connected to keratinocyte proliferation and migration.[31] Ultimately, this temporary structure is replaced, as the wound matures, by the addition of protein molecules: proteoglycans and collagen. HA molecules are able to absorb

Table 1. Percentage Healing Index (PHI) and Pain Index (PI) Scores Obtained in the Study Group (SG) and Control Group (CG) for Every Patient Gender, Age, and T_0 and T_1 Anatomical Area Are Reported

Patient number SG	*Gender*	*Age*	*Anatomical area*	*Wound area SG T_0*	*Wound area SG T_1*	*PHI SG*	*PI SG*	*Patient number CG*	*Gender*	*Age*	*Anatomical area*	*Wound area SG T_0*	*Wound area SG T_1*	*PHI CG*	*PI CG*
			Small wounds (area < 19.625 mm^2)								*Small wounds (area < 19.625 mm^2)*				
1	F	33	Tongue apex	1.39	0	100	1	1	F	61	Tongue margin	3.12	1.20	61.54	2
2	M	64	Palate	7.50	5.60	25.33	3	2	M	30	Upper lip	3.87	2.50	35.4	2
3	F	57	Adherent gingiva	7.80	2.20	71.8	2	3	F	50	Palate	8.75	7.60	13.14	3
4	M	41	Lower lip	8.31	5.62	32.37	2	4	F	42	Tongue dorsum	8.90	6.80	23.6	3
5	M	32	Palate	8.87	1.06	88.05	4	5	F	64	Palate	9.30	4.50	51.6	2
6	F	29	Lower lip	9.70	3.40	64.95	3	6	F	64	Tongue dorsum	12.06	5.50	54.4	3
7	M	30	Tongue dorsum	10.40	0	100	2	7	M	48	Lower lip	16.80	7.90	53.0	3
8	F	39	Upper lip	12.60	3.80	69.84	2								
9	F	69	Tongue apex	14.60	0	100	1								
10	M	42	Tongue apex	17.00	3.70	78.24	3								
11	M	51	Lower lip	18.50	8.60	53.51	3								
			Large wounds (area > 19.625 mm^2)								*Large wounds (area > 19.625 mm^2)*				
12	F	37	Palate	20.20	19.00	5.95	2	8	F	35	Lower lip	24.8	2.03	91.8	1
13	M	42	Alveolar mucosa	21.00	0	100.0	3	9	F	35	Cheek mucosa	27.90	3.20	88.53	3
14	M	31	Tongue dorsum	21.30	7.80	63.38	3	10	M	36	Tongue dorsum	28.00	18.40	34.29	4
15	M	27	Lower lip	27.70	4.10	85.20	3	11	M	27	Cheek mucosa	31.25	5.50	82.4	2
16	F	70	Tongue apex	28.00	9.10	67.5	2	12	F	29	Cheek mucosa	32.60	19.80	39.26	4
17	M	37	Tongue margin	28.90	6.00	79.24	2	13	M	59	Retrocommissural area	43.90	33.00	24.83	3
18	F	51	Tongue apex	32.00	19.20	40.00	2	14	M	42	Lower lip	52.20	25.30	51.53	4
19	M	40	Cheek mucosa	32.70	5.90	81.96	3	15	M	49	Cheek mucosa	55.75	16.50	70.4	2
20	M	55	Cheek mucosa	34.20	3.60	89.48	3	16	F	57	Retrocommissural area	58.75	12.50	78.72	1
21	F	28	Lower lip	34.50	17.30	49.86	4	17	M	39	Tongue dorsum	63.00	62.25	1.2	3
22	F	59	Cheek mucosa	37.50	30.75	18.0	1	18	M	36	Cheek mucosa	92.75	87.00	6.2	2
23	M	42	Retrocommissural area	37.70	7.30	80.64	2								
24	M	51	Tongue margin	38.50	10.75	72.08	2								
25	M	47	Retrocommissural area	42.60	28.82	32.35	3								
26	F	38	Cheek mucosa	47.20	13.40	71.61	3								
27	F	54	Palate	49.00	21.00	57.14	3								
28	M	66	Cheek mucosa	52.00	17.00	67.31	4								
29	F	62	Cheek mucosa	53.00	5.60	89.43	4								
30	F	52	Cheek mucosa	54.50	34.00	37.62	5								
31	F	56	Cheek mucosa	103.25	79.50	23.01	3								

up to 3000 times their own weight in water,[31] allowing for control of hydration during wound repair. The presence of elevated HA levels during this process is also of particular relevance to cell proliferation and migration. Inn part because of HA's presence, cell anchorage to the extracellular matrix is weakened, permitting detachment and facilitating cell migration and division.[32,33]

A gel based on amino acids and sodium hyaluronate has been recently used to promote wound healing in the oral cavity. The used compound contains L-proline, L-leucine, L-lysine, glycine, and sodium hyaluronate. Colella et al. recently showed, in an *in vitro* study, which mechanisms could be behind the wound healing improvement. They investigated the molecular and functional changes induced in keratinocytes by sodium hyaluronate alone and in combination with synthetic amino acids, discovering that HA can induce changes in the expression of genes related to a migratory phenotype in keratinocytes by modulating extracellular proteolysis, cytoskeleton, and metabolism; and that the addition of synthetic amino acids (L-proline, L-leucine, L-lysine, and glycine) produces a distinct gene-expression profile in keratinocytes.[34]

As a consequence, the product is supposed to influence the tissue repairing process after laser surgical procedures, thereby leading to faster re-epithelialization of the wound area, better functional recovery, and less postoperative pain.

Concerning the area of the lesion, SG patients displayed significantly reduced PHI scores 1 week after surgery. This suggests that the experimental postoperative protocol, involving the application of the amino acid-HA compound, possibly produced faster healing in the postsurgical wound area. This was especially true for small wound areas; in fact, when taking into account lesions whose area was larger than a wound of 5 mm diameter ($19.625\,mm^2$), the average PHI after 7 days did not significantly differ between SG and CG. Statistically significant difference still remained when comparing wounds in SG and CG $< 19.625\,mm^2$. This result could possibly be the consequence of the same quantity of product applied for small and large lesions, resulting in a smaller topical concentration of amino acids and HA for larger wounds. Statistical power testing revealed that a small significant difference in PHI score, such the one detected among the lesions $< 19.625\,mm^2$, could not be easily revealed from this experiment, because it had only a 30% power to detect a difference between means of 16.41 with a significance level (α) of 0.05 (two tailed).

The calculation of the area of the lesion (measured in mm^2) at T_0 and T_1 is a precise and reliable measurement method, with the further advantages of being easily applicable and inexpensive. This method allows the clinician to verify the actual reduction of the lesion over time, which could possibly be used for several measurements in dentistry or biology. Area calculation is achieved only through accurate clinical photographs, a bloodless field, and measurements of wound values obtained with specific computer software.

The results of our study are generally in line with those described in literature. A recent systematic review on the effects of HA derivatives and their effect on wound healing revealed that in the considered studies, sodium hyaluronate speeds up the healing process in burns, epithelial surgical wounds, and chronic wounds from diabetes or venous insufficiency.[33] Another previous study regarding the same compound used to promote laser oral wound healing had concluded that HA and amino acids had improved the healing time of the laser wounds. In contrast with this study, we did not observe any complication in the CG or in the SG.[35] Experimental conditions were slightly different, as in this study, oral benign lesions were excised using a diode laser and lesion size was $\sim$20 mm for all the lesions in both the experimental and control groups. Also wound healing speed measurement was different, as in the study of Favia et al.,[35] the considered parameter were the days before complete healing, whereas in our study the measurement were repeated at a standard time (7 days).

A pilot study from Vescovi et al. had showed that HA led to less pain perception in the immediate postoperative period and 3 days after laser surgery for oral biopsies. In this study, 20 patients underwent excisional biopsies of oral benign lesions, and patients gave perceived pain evaluation through three different pain scales.[36] Pain perception was reported to be less in the compound-treated sample, when compared with a control group of previously treated patients. In this study, no area measurement was made, nor was statistical testing available. Results concerning pain perception vary considerably from our study; in fact the use of an amino acid and HA compound led to slight reductions in pain as reported by the patients. The values were, however, statistically nonsignificant, as the difference between the two groups was only 0.38 (on the NRS scale). Therefore, the quantification of pain experience after a surgical procedure of comparable extent may be very different, as it depends not only on the technique used or the product applied but also on the emotional perception of each individual. In fact, individual sensitivity, position in the oral mucosa, and size of the lesion may have a greater impact on pain perception than does the administration of sodium hyaluronate. Furthermore, PI power testing revealed that the size of the samples was too small to detect a relevant difference in PI (for example, 0.5) as experiment had a 40% power to detect a difference between means of 0.48 with a significance level (α) of 0.05 (two tailed).

Nevertheless, this study has several limitations that must be taken into account. It was not possible to measure the volume of removed tissue in order to consider also the depth of the wounds, which also has importance in the healing time. This is a common limitation of other animal and human studies, and measurement of wound area is generally considered an objective method to monitor wound healing. It was not possible to monitor the healing process every day; therefore, it is not possible to know whether differences in healing speed take place in the first days or later in the healing period. Moreover, group composition resulted in being unbalanced when taking into account small and large lesions divided by gender; for instance, when considering small wounds in SG, there were 6 males and 5 females, while in CG there were 2 males and 5 females. This is because the division between large size and small size wounds was done after the result collection. Finally, wound locations varied considerably; there are nine different locations for SG, and seven different locations for CG. Even if the location of the wound plays a role in healing time and pain perception, it was decided not to take into account the anatomical areas, as this would have meant dividing the samples into a large number of smaller samples of unequal number, which would have been of small statistical power.

Conclusions

In conclusion, the results showed that the application of a compound consisting of a group of amino acids and sodium hyaluronate on surgical wounds could considerably quicken the repair processes. The same was not observed for postoperative pain, on which the compound did not have any measurable effect. Nevertheless, the reduction in perceived pain may also be considered an indirect indicator of the improved healing process in postoperative wounds, even if no significant differences were found between groups. The conclusions of this study can only be applied to the specific experimental conditions, with no or little knowledge about lesions larger than those taken into account in the experimental and control groups.

The use of the compound can be recommended in all cases in which laser surgical procedures cause lesion healing via secondary intention in the mouth, which is certainly more subject to continuous trauma because of the normal functions of the oral cavity. Further studies are required to better understand in which time interval the healing speed is higher, in the first days or later, and if a higher local concentration of the compound could accelerate the healing time of even large laser wounds.

Author Disclosure Statement

Errekappa Euroterapici provided the fees for the manuscript editing service. Dr. Gaspare Palaia and Dr. Gianluca Tenore received research funding from Errekappa Euroterapici.

The other authors have no relevant financial or nonfinancial relationships to disclose.

References

1. Pick, R.M., and Colvard, M.D. (1993). Current status of lasers in soft tissue dental surgery. J. Periodontol. 64, 589–602.
2. Goharkhay, K., Moritz, A., Wilder-Smith, P., et al. (1999). Effects on oral soft tissue produced by a diode laser in vitro. Lasers Surg. Med. 25, 401–406.
3. Eversole, L.R. (1997). Laser artifacts and diagnostic biopsy. Oral Surg. Med. Oral Pathol. Oral Radiol. Endod. 83, 639–640.
4. Romanos, G.E., Pelekanos, S., and Strub, J.R. (1995). A comparative histological study of wound healing following Nd:YAG laser with different energy parameters and conventional surgical incision in rat skin. J. Clin. Laser Med. Surg. 13, 11–16.
5. Romanos, G.E., Pelekanos, S., and Strub, J.R. (1995). Effects of Nd:YAG laser on wound healing processes: clinical and immunohistochemical findings in rat skin. Lasers Surg. Med. 16, 368–379.
6. West, D.C., Hampson, I.N., Arnold, F., and Kumar, S. (1985). Angiogenesis induced by degradation products of hyaluronic acid. Science 228, 1324–1326.
7. Sattar, A., Rooney, P., Kumar, S., et al. (1994). Application of angiogenic oligosaccharides of hyaluronan increases blood vessel numbers in rat skin. J. Invest. Dermatol. 103, 576–579.
8. Trochon, V., Mabilat–Pragnon, C., Bertrand, P., et al. (1997). Hyaluronectin blocks the stimulatory effect of hyaluronan-derived fragments on endothelial cells during angiogenesis in vitro. FEBS Lett. 418, 6–10.
9. Deed, R., Rooney, P., Kumar, P., et al. (1997). Early-response gene signalling is induced by angiogenic oligosaccharides of hyaluronan in endothelial cells. Inhibition by non-angiogenic, high-molecular-weight hyaluronan. Int. J. Cancer 71, 251–256.
10. Slevin, M., Krupinski, J., Kumar, S., et al. (1998). Angiogenic oligosaccharides of hyaluronan induce protein tyrosine kinase activity in endothelial cells and activate a cytoplasmic signal transduction pathway resulting in proliferation. Lab. Invest. 78, 987–1003.
11. Slevin, M., Kumar, S., and Gaffney, J. (2002). Angiogenic oligosaccharides of hyaluronan induce multiple signaling pathways affecting vascular endothelial cell mitogenic and wound healing responses. J. Biol. Chem. 277, 41,046–41,059.
12. Turley, E.A., Austen, L., Vandeligt, K., et al. (1991). Hyaluronan and a cell-associated hyaluronan binding protein regulate the locomotion of ras-transformed cells. J. Cell. Biol. 112, 1041–1047.
13. Mesa, F.L., Aneiros, J., Cabrera, A., et al. (2002). Antiproliferative effect of topic hyaluronic acid gel: study in gingival biopsies of patients with periodontal disease. Histol. Histopathol. 17, 747–753.
14. Iocono, J.A., Krummel. T.M., Keefer, K.A., et al. (1998). Repeated additions of hyaluronan alters granulation tissue deposition in sponge implants in mice. Wound Repair Regen. 6, 442–448.
15. Kielty, C.M., Whittaker, S.P., Grant, M.E., et al. (1992). Type IV collagen microfibrils: evidence for a structural association with hyaluronan. J. Cell Biol. 118, 979–990.
16. Rooney, P., and Kumar, S. (1993). Inverse relationship between hyaluronan and collagens in development and angiogenesis. Differentiation 54, 1–9.
17. Scully, M.F., Kakkar, V.J., Goodwin, C.A., et al. (1995). Inhibition of fibrinolytic activity by hyaluronan and its alcohol ester derivatives. Thromb. Res. 78, 255–258.
18. West, D.C., Shaw, D.M., Lorenz, P., et al. (1997). Fibrotic healing of adult and late gestation fetal wounds correlates with increased hyaluronidase activity and removal of hyaluronan. Int. J. Biochem. Cell Biol. 29, 201–210.
19. Huang-Lee, L.L., and Nimni, M.E. (1993). Fibroblast contraction of collagen matrices with and without covalently bound hyaluronan. J. Biomater. Sci. Polym. Ed. 5, 99–109.
20. Mariggiò, M.A., Cassano, A., Vinella, A., et al. (2009). Enhancement of fibroblast proliferation, collagen biosynthesis and production of growth factors as a result of combining sodium hyaluronate and aminoacids. Int. J. Immunopathol. Pharmacol. 22, 485–492.
21. Walsh, J.T., Jr., Flotte, T.J., and Deutsch, T.F. (1989). Er:YAG laser ablation of tissue: effect of pulse duration and tissue type on thermal damage. Lasers Surg. Med. 9, 314–326.
22. Araki, A.T., Ibraki, Y., Kawakami, T., and Lage–Marques, J.L. (2006). Er:Yag laser irradiation of the microbiological apical biofilm. Braz. Dent. J. 17, 296–299.
23. Lopes, B.M., Theodoro, L.H., Melo, R.F., Thompson, G.M., and Marcantonio, R.A. (2010). Clinical and microbiologic follow-up evaluations after non-surgical periodontal treatment with erbium:YAG laser and scaling and root planing. J. Periodontol. 81, 682–691.
24. Hamamci, N., Akkurt, A., and Başaran, G. (2010). In vitro evaluation of microleakage under orthodontic brackets us-

ing two different laser etching, self etching and acid etching methods. Lasers Med. Sci. 25, 811–816.
25. Catone, G.A., and Alling, C.C. (1997). Laser application in oral and maxillofacial surgery. Philadelphia: W.B. Saunders.
26. Pick, R.M., and Colvard, M.D. (1993). Current status of lasers in soft tissue dental surgery. J. Periodontol. 64, 589–602.
27. Romeo, U., Palaia, G., Botti, R., Leone, V., Rocca, J.P., and Polimeni, A. (2009). Non-surgical periodontal therapy assisted by potassiumtitanyl- phosphate laser: a pilot study. Lasers Med. Sci. 25, 891–899.
28. Romeo, U., Del Vecchio, A., Ripari, F., et al. (2007). Effects of different laser devices on oral soft tissues: in vitro experience. J. Oral Laser Appl. 7, 155–159.
29. Romeo, U., Palaia, G., Del Vecchio, A., et al. (2010). Effects of KTP laser on oral soft tissues. An in vitro study. Lasers Med. Sci. 25, 539–543.
30. Gill, D. (1998). Angiogenic modulation. J. Wound Care. 7, 411–414.
31. Bourguignon, L.Y.W., Ramez, M., Gilad, E., et al. (2006). Hyaluronan-CD44 interaction stimulates keratinocyte differentiation, lamellar body formation/secretion, and permeability barrier homeostatis. J. Invest. Dermatol. 126, 1356–1365.
32. Culp, L.A., Murray, B.A., and Rollins, B.J. (1979). Firbronectin and proteoglycan as determinants of cell-substratum adhesion. J. Supramol. Struct. 11, 401–427.
33. Voigt, J., and Driver, V.R. (2012). Hyaluronic acid derivatives and their healing effect on burns, epithelial surgical wounds, and chronic wounds: a systematic review and meta-analysis of randomized controlled trials. Wound Repair Regen. 20, 317–331.
34. Colella, G., Vicidomini, A., Soro, V., Lanza, A., and Cirillo, N. (2012). Molecular insights into the effects of sodium hyaluronate preparations in keratinocytes. Clin. Exp. Dermatol. 37, 516–520.
35. Favia, G., Mariggio, M.A., Maiorano, F., Cassano, A., Capodiferro, S., and Ribatti, D. (2008). Accelerated wound healing of oral soft tissues and angiogenic effect induced by a pool of aminoacids combined to sodium hyaluronate (AMINOGAM). J. Biol. Regul. Homeost. Agents 22, 109–116.
36. Vescovi, P., Manfredi, M., Ripasarti, A., et al. (2013). Effects of amino acids and sodium hyaluronate gel (AMINOGAM®) in the post operative period after surgical laser treatment of oral lesions: a pilot study. www.docstoc.com/docs/47804854/EFFECTS-OF-AMINO-ACIDS-AND-SODIUM-HYALURONATE-GEL-AMINOGAM-IN (Last accessed August 17, 2013).

Address correspondence to:
Umberto Romeo
Department of Oral Sciences
"Sapienza" University of Rome
Via della Brianza, 16
Via Caserta 6
00161 Rome
Italy

E-mail: umberto.romeo@uniroma1.it