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EFFECT OF TOPICAL MELATONIN LOADED GELATIN SPONGE ON PALATAL WOUND HEALING (RANDOMIZED CONTROLLED CLINICAL TRIAL)

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ABSTRACT

INTRODUCTION: For many muco-gingival deformities, autogenous soft tissue grafts are the gold standard treatment. The palate is the most typical site for soft tissue graft harvesting. Epithelialized graft harvesting technique is one of the most efficient. Yet, it leaves an open palatal wound that is only healed by secondary intention. Many dressing materials have been used -to cover and protect the donor site- either alone or combined with other biological materials as: non-eugenol and eugenol dressings, Collagen, hydrogels and, resin-based dressings. Melatonin is well known for its anti-oxidant and anti-inflammatory effects; it has the ability to decrease the effects of various pro-inflammatory mediators. It can scavenge free radicals and reduce oxidative stress, which is often associated with inflammation. Melatonin's ability to regulate the immune response and dampen the pro-inflammatory cascade has gained interest for potential therapeutic applications.

Study objective: To assess the effect of topically applied melatonin loaded gelatin sponge on palatal wound healing.

MATERIALS AND METHODS: Twenty-six surgical sites for free palatal graft procurement were included in the study. They were equally divided into two groups: Test group, the palatal donor site was covered by topical Melatonin loaded gelatin sponge. Control group, the site was covered by carbopol loaded gelatin sponge. Wound healing was evaluated using photo-digital planimetry on the day of surgery and, at seven days post-surgical. Healing index of Landry was used at the seventh day. Pain was assessed via VAS for a week from the day of surgery.

RESULTS: Photo-digital planimetry showed that the test group exhibited more reduction in the percentages of wound area than control although it was not statistically significant. Healing index of Landry revealed no statistically significant difference between the two groups. No significant differences in VAS scores between the two groups.

CONCLUSION: Melatonin could be beneficial in improving palatal wound healing.

KEYWORDS: Gelatin sponge, melatonin, palatal wound healing, tissue harvesting.

RUNNING TITLE: Melatonin in palatal wound healing.

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INTRODUCTION

Muco-gingival deformities have an impact on both esthetics and function[1]. Lack of keratinized mucosa and gingival recession are claimed to be the most prevalent muco-gingival problems [1]. The optimal treatment option for these conditions is soft tissue grafting [2].

Soft tissue grafting have become indispensable in periodontal plastic surgeries in order to correct mucogingival defects [2]. Despite the advances in materials used as alternatives to harvesting soft tissue grafts, autogenous grafts are deemed to be the gold standard up till now.

Absence of keratinized tissue around implants predisposes to peri-implant mucositis [3]. Furthermore, teeth with narrow zones of keratinized tissue have been observed to be more prone to recession [4].

Several harvesting procedures have been utilized; trap door, single incision and epithelialized graft harvesting approaches. Although the first two techniques allow healing with primary intention [5], they are difficult to execute and can interrupt the blood supply to the overlying flap causing necrosis [5]. Contrarily , epithelialized graft harvesting, is a

more reliable technique, since it involves the dense and firm lamina propria layer [6]. Despite the benefits obtained from this technique in dealing with muco-gingival deformities, it is not without limitations; it creates an open palatal wound that heals by secondary intention, resulting in increased discomfort and post-operative morbidity [7]. To overcome those inadequacies, various dressing materials were tested [8, 9].

Traditional dressing materials promote wound healing solely through insulating the wound area from external noxious assaults. Yet, they are dry and may cause pain since they adhere to the wound [10]. Recent achievements concerned with wound healing have led to invention of different types of wound dressings.

Biological wound dressings provide mechanical and physiological effects. They hasten wound healing by modulating many aspects of the healing process [10]. These include: amnion and chorion membranes[11], platelet rich fibrin (PRF)[12], and collagen [13]. However, The search for an optimal material that enhances wound healing is still ongoing, and no gold standard has yet been achieved [8, 9].

Melatonin (N-Acetyl-5-Methoxy Tryptamine), a hormone secreted by pineal gland could serve as a beneficial post-surgical treatment [14, 15]. It has a potent antioxidant and anti-inflammatory actions in the oral cavity where it reaches there via saliva [16]. It significantly improves wound healing and collagen maturation and deposition [17, 15].

Melatonin down-regulates the influence of several hallmarks of pro- inflammatory mediators, including interleukin (IL)-1ß and IL-6 by *blocking* nuclear factor kappa B (NF-κB) binding to nuclear DNA [18]. The use of this promising material has been attempted in many experimental trials, Vorotelyak et al. [19] assessed the effect of melatonin on the rate of wound closure under in vitro conditions, and the effect of the same material on the rate of wound healing under in vivo conditions. They assumed that melatonin stimulates epithelial migration in wound models both in vitro and vivo.

In another study [15], researchers created an injectable melatonin-loaded hydrogel and investigated effect on skin wounds in full thickness incisions. They demonstrated that the rate of healing in the melatonin-loaded gel group was greater when compared to using the hydrogel alone.

Melatonin has been demonstrated to improve wound healing in diabetic rats [17]. It also prevented oral radiation-induced mucositis [20].

Therefore, the purpose of this study was to investigate if topical melatonin loaded hydrogel application would improve the course of palatal wound healing following graft harvesting.

The null hypothesis of this study was that there would be no significant difference in the healing rate between melatonin-loaded gelatin sponge and control group.

MATERIALS AND METHODS

2.1 Sample size and characterization:

Sample size was estimated assuming 5% alpha error and 80% study power. Şener et al. [21] reported mean ± SD= 98 ± 1 percentage wound closure after 14 days when melatonin was used and = 94 ± 6 when melatonin was not used. Based on comparison of means, sample size was calculated to be 12 per group, increased to 13 to make up for cases lost to follow up. The total sample size required= number of groups × number per group= 2 x 13 = 26 [22].

The primary outcome of this clinical trial was assessing palatal wound healing through photo-digital planimetry.

The secondary outcome was assessing pain perception through visual analogue scale (VAS) and wound healing using healing index of Landry.

2.2 Study design and population

Twenty-six patients participated in this randomized controlled clinical trial in two parallel study groups. Patients were recruited from the Faculty of Dentistry, Alexandria University, outpatient clinic at the Department of Oral Medicine, Periodontology, Oral Diagnosis and Oral Radiology. The PICOT question was: Did patients needing autogenous palatal grafts (P: population), receiving a melatonin loaded gelatin sponge over the palatal wounds (I: intervention) compared to placebo dressing (C: control), show advanced healing (O: outcome) at one week interval post-surgery (T: time)?

2.3 Inclusion and exclusion criteria:

Patients were eligible, if they fulfilled the following criteria: Age <50 years, systemically healthy, palatal tissue thickness ≥3mm, using University of North Caroline (UNC-15) periodontal probe (Medesy, Maniago, Italy), and having a muco-gingival deformity (such as lack of attached gingiva or gingival recession) that requires harvesting an autogenous graft. Patients were excluded if they were cigarette, pipe, water pipe smokers or indulged in any deleterious habits that may affect palatal wound healing. Pregnant or lactating women, patients who had palatal infection or were under any medical treatment that could affect soft tissue healing were also excluded from the study.

2.4 Ethical approval and registration:

Study was conducted in accordance to the principles of the modified Helsinki code for human clinical studies (2013) [23], and was approved by the Ethics Committee of faculty of dentistry, Alexandria University **(IRB 00010556)- (IORG 0008839)**. The purpose and

nature of the study were explained to the patients and an informed consent obtained from study participants prior to any intervention. The study complied with CONSORT® guidelines for the conduct of randomized controlled clinical trials [24]. (Figure1).

2.5 Randomization:

Participants complying with the inclusion criteria were randomly assigned using a computer-generated list of random numbers [25] either to test or control group. Allocation was performed in blocks of 4 by a trial independent individual and the allocation ratio was intended to be equal. The operators assessing results was blinded to the material used.

2.6 Gel preparation:

Melatonin*loaded carbopol hydrogel involved dissolving 1 gram of carbopol in 100 milliliters of deionized water with stirring at 600 rpm and 25°C. Then, 3 grams of melatonin dissolved in 1 milliliter of ethanol were added to the formed gel while stirring at 600 rpm and 25°C. The pH of the mixture was adjusted to 7.4 using triethanolamine until a gel consistency was achieved[26].

Carbopol hydrogel involved dissolving 1 gram of carbopol in 100 milliliters of deionized water with stirring at 600 rpm and 25°C. The pH of the mixture was then adjusted to 7.4 using triethanolamine until a gel consistency was obtained[27].

2.7 Surgical procedures:

Phase I therapy was established to all patients, in order to achieve optimal plaque control and gingival health conditions (O'Leary plaque index < or =15%, gingival index ≅ 0). Surgeries were performed by the same operator (SN). Autogenous free palatal grafts were procured applying Sullivan and Atkins classical approach [28].

The width and the length of the graft were measured by a standard UNC-15 periodontal probe* to the nearest millimeter. After harvesting the free palatal grafts from the palatal donor sites, an adrenaline-soaked sterile surgical gauze was applied to the palatal wounds and pressure was maintained for 5 minutes to control the bleeding, then either a carbopol or melatonin based topical formula loaded gelatin sponge was applied to the donor sites. Flowable composite† was used as a stent; after spot etching the palatal surface of the teeth adjacent to the donor site, the teeth were rinsed and dried. Subsequently, a drop of bonding agent was applied, thinned out using air, and then cured with light. Finally, flowable composite was used to cover the entire sponge, including the embrasures between the teeth[29].

2.8 Postsurgical care:

Immediately following the procedure, an ice pack was administered, and post-operative instructions were given (both written and verbal). A standardized analgesic was prescribed to the patients: Ibuprofen (Brufen; Kahira Pharm. & Chem. Ind. Co., Egypt, under license of: Abbott Laboratories– USA) (600 mg up to 3 tablets/day). All patients were instructed to rinse with 0.12% chlorhexidine gluconate (Hexitol; The Arab Drug Company (ADCO)-Egypt) mouth rinse twice per day, for two weeks starting 24 hours after the surgical procedure. Patients were recalled after one week for post-operative follow-up. Clinical follow-up photographs were taken to measure the healing progress and ensure reproducibility.

2.9 Post-surgical assessment:

Photo-digital planimetry:

Digital Single-Lens Reflex (DSLR)(Nikon-D5300) camera body, a macro lens, and a mounted ring flash were used to obtain a high-quality photograph. Surface reflecting intra-oral mirrors were used to obtain a line of sight directly *perpendicular* to the palatal wound site to avoid any distortion to the wound in the photograph that could affect the measurements[30].

Clinical follow-up photographs included a *standard-sized* visual scale placed near to the wound at the day of surgery and after one week, photographs were imported into a computer software (ImageJ 1.53k) to measure the healing progress. Tracing of the remaining wound area was completed by two independent examiners, blinded to nature of the administered gel. (Figure 2).

The remaining wound area was traced on ImageJ‡ using a mouse based on the following criteria:

- The wound edges were traced by identifying the fibrinous tissue, fibrin, slough, scab, granulation or necrotic tissue which was not covered by epithelium and appeared to have a different color and /or tissue texture match when compared with the adjacent unwounded area.
- Immature re-epithelized areas around the wound edges, tissue undergoing the process of remodeling and with the appearance of erythema due to inflammation, was not considered as wound remaining area[31].

Inter- and intra-examiner reliability were calculated and intraclass correlation coefficient (ICC) ranged from 0.81 to 0.93 indicating good to excellent agreement between examiners and across time[32].

Healing index of Landry[33]:

This index was used to assess the quality of the healed tissue after seven days from the day of surgery**.** The index uses a score ranging from 1 to 5, where 1 indicates (very poor) healing and 5 indicates

* Baoji GuoKang Bio-technology Co., Ltd China.
* Medesy, Maniago, Italy.
† Nexcomp Flow, Meta Biomed, Korea.

‡ The National Institutes of Health, USA http://imagej.nih.gov/ij/

(excellent) healing. The index evaluates the parameters of tissue colour, bleeding response to palpation, presence of granulation tissue, characteristics of the incision margins, and the presence of suppuration.

Pain assessment:

Patients were asked to grade the severity of their pain on a VAS[34] from the day of the operation and for a week. The patients recorded the score every day for 7 days.

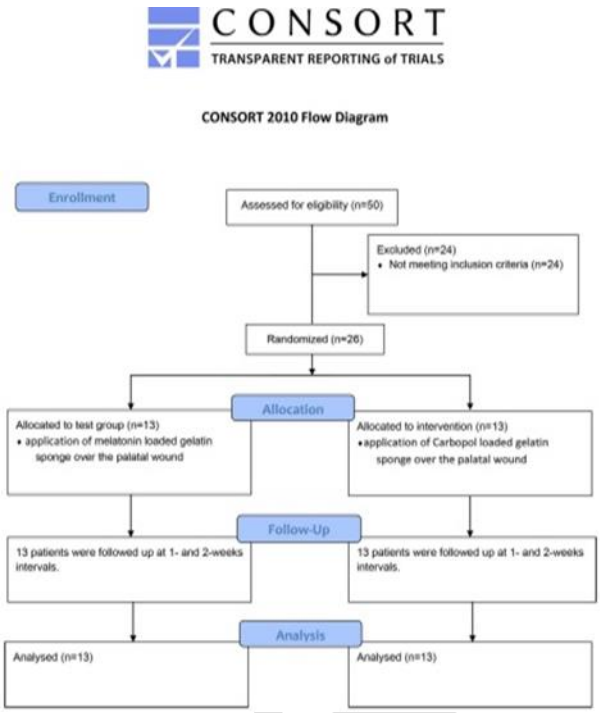

Figure (1): Randomized controlled clinical trial flowchart for the study

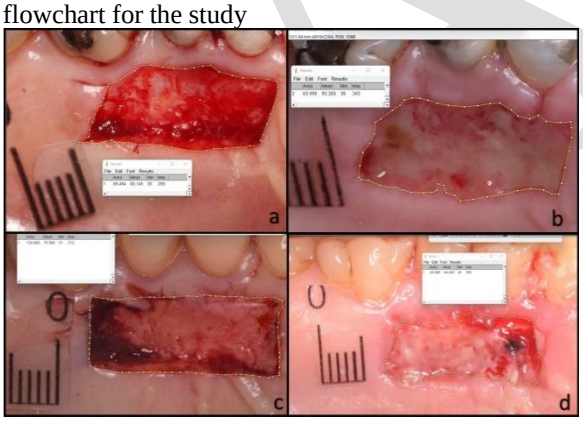

Figure (2): Wound area, photo-digital planimetry: (a, b) control group: a, the day of surgery b, after one week. (c, d) test group: c, the day of surgery d, after one week

Statistical analysis:

Normality was tested for all variables using descriptive statistics, plots (Q-Q plots and histogram), and normality tests. Means and standard deviation (SD) were calculated for quantitative normally distributed variables, in addition to median and interquartile range (IQR) for non-normally distributed variables. Frequencies and percentages were calculated for qualitative variables. Comparisons between the two study groups were done using independent samples t-test for quantitative normally distributed variables (age, graft thickness, length, and width), and using Mann-Whitney U test for non-normally distributed variables (digital planimetry results). Significance was set at p value <0.05. Data were analyzed using IBM SPSS for Windows (Version 23.0).

RESULTS

3.1 Sample characterization:

Patients were equally distributed between the two study groups, 13 patients per group. No statistically significant inter-group differences were observed regarding age, gender, and location of the donor site. (Table 1).

3.2 Graft dimensions:

The mean width of the graft was 7.25 (±1.42) mm and 8.38 (±1.85) mm for test and control groups, respectively. The mean length of the graft was 15.46 (±4.20) mm and 14.23 (±4.21) mm for test and control group, respectively. As for the graft thickness, it was 1.58 (±0.19) mm for test group and 1.54 (±0.14) mm for control. Statistical analysis revealed no significant difference between the two groups regarding the dimensions of the grafts used. (Table 2).

3.3 Photo-digital planimetry:

Melatonin group presented with a mean initial area of 86.36 (±46.71) mm. this surface area decreased to 69.86 (±35.99) mm after one week. For the carbopol group the initial mean area was 106.16 (±68.59) and it diminished to 81.07 (±53.13) mm after the first week, respectively. There was no statistically significant difference between the two groups ($p \geq 0.05$). (Table 3).

3.4 Healing index of Landry:

There was no statistically significant difference between both groups ($p \geq 0.05$).

In the control group, out of the total number of sites evaluated, 2 showed (good) healing, 10 showed (poor) healing, and 1 showed (very poor) healing. In the test group, 4 sites showed (good) healing, 9 sites showed (poor) healing, and no sites had (very poor) healing. (Table 4).

3.5 Pain assessment (VAS):

There was no statistically significant difference in reduction of pain between the two groups ($p \geq 0.05$). The test group showed ***higher*** but non-significant pain reduction in all time points. (Table 5).

Table 1: Baseline characteristics of the two study groups.

		Test (n=13)	Control (n=13)	P value
Age [a]	**Mean (SD)**	31.00 (6.43)	30.92 (11.25)	0.98
Gender: n (%)[b]	**Male**	5 (38.5%)	5 (38.5%)	1.00
	Female	8 (61.5%)	8 (61.5%)	
Location: n (%)[c]	**Right**	7 (53.8%)	2 (15.4%)	0.10
	Left	6 (46.2%)	11 (84.6%)	

[a] independent samples t-test, [b] Chi-square test, [c] Fisher exact test were used

Table 2: Graft thickness, length, and width in the two study groups

	Test (n=13)	Control (n=13)	T-test P value
	Mean (SD)		
Graft thickness(mm)	1.58 (0.19)	1.54 (0.14)	0.56
Graft length(mm)	**15.46 (4.20)**	**14.23 (4.21)**	**0.46**
Graft width(mm)	**7.25 (1.42)**	**8.38 (1.85)**	**0.10**

Table 3: Digital planimetry of Initial and remaining wound surface area in the two study groups.

			Test (n=13)	Control (n=13)	MWU p value
Initial Wound Surface area (mm)		Mean (SD)	86.36 (46.71)	106.16 (68.59)	0.34
Remaining Wound Surface area	1 week	Mean (SD)	69.86 (35.99)	81.07 (53.13)	0.92

MWU: Mann-Whitney U.

Table 4 : Comparison of healing index by Landry between the two study groups

	Test	Control	MWU P value
Very poor (1)	0 (0%)	1 (7.7%)	0.39
Poor (2)	9 (69.2%)	10 (76.9%)	
Good (3)	4 (30.8%)	2 (15.4%)	
Very good (4)	0 (0%)	0 (0%)	
Excellent (5)	0 (0%)	0 (0%)	

Table 5: Pain assessment using VAS in the two study groups

		Test (n=13)	Control (n=13)	MWU p value
Day 1	**Mean (SD)**	65.38 (19.84)	67.69 (22.42)	0.61
	Median (IQR)	60.00 (35.00)	70.00 (40.00)	
Day 2	**Mean (SD)**	56.15 (19.38)	59.23 (24.31)	0.58
	Median (IQR)	50.00 (35.00)	60.00 (45.00)	
Day 3	**Mean (SD)**	47.69 (20.88)	48.46 (21.93)	0.84
	Median (IQR)	50.00 (40.00)	40.00 (30.00)	
Day 4	**Mean (SD)**	37.69 (20.88)	43.08 (22.13)	0.51
	Median (IQR)	40.00 (40.00)	40.00 (35.00)	
Day 5	**Mean (SD)**	28.46 (20.76)	33.85 (23.29)	0.58
	Median (IQR)	30.00 (35.00)	30.00 (35.00)	
Day 6	**Mean (SD)**	21.54 (19.08)	27.69 (20.88)	0.48
	Median (IQR)	20.00 (35.00)	20.00 (25.00)	
Day 7	**Mean (SD)**	14.62 (17.61)	20.00 (18.26)	0.39
	Median (IQR)	10.00 (25.00)	10.00 (20.00)	

*Statistically significant at p value <0.05

DISCUSSION

Autogenous soft tissue grafts are crucial treatment for many muco-gingival deformities. Palatal mucosa is the common site for graft harvesting. The most commonly used surgical technique involves harvesting of an epithelialized soft tissue graft from the palatal mucosa and then transplantation into the recipient site.

Post operative pain and patient morbidity are the most commonly reported drawbacks. There are numerous approaches to treat the donor site following soft tissue grafting to avoid the morbidity associated with the raw area.

Traditional wound dressings including natural or synthetic bandages, cotton wool, and gauzes with varied degrees of absorption were utilized in the past. Their main role was to keep the wound dry by enabling wound exudates to evaporate and preventing harmful microorganisms from entering the wound[10]. Yet, it has recently been demonstrated that a moist wound environment promotes faster and better wound healing[10].

In this study, a gelatin sponge was used as a carrier for the gels; it had no inherent hemostatic properties and therefore served as an inert medication carrier. The sponge was made of pure collagen protein with a neutral PH that is intensely porous in structure[13]. With all those properties, it proved to be the perfect choice to use.

Melatonin exerts anti-oxidant and anti-inflammatory effects[26, 35]. It has been demonstrated that melatonin had a positive effect in treatment of periodontitis, osseointegration of dental implants, and bone regeneration[35]. In this trial we assessed the impact of melatonin gel on the healing of palatal wound.

Melatonin gel was used in a concentration of 3% based on a study by Abdel Moneim et al. [26] , who performed a dose-response study on melatonin gel and the maximal therapeutic effect was obtained with 3% melatonin gel.

Carbopol -a type of synthetic polymer- is generally considered inert and does not have a significant chemical interaction with the ingredients of a gel formulation[36].

It is primarily used as a gelling agent; it provides excellent muco-adhesion effect[37]. It can maintain its characters at PH of 7.4 -which is the physiological PH of saliva- thus minimizing irritation to oral mucosa[37]. Based on this, it was an ideal choice to use carbopol as a gelling agent in the inert gel formulation as in placebo group, or loaded with melatonin as in the test group.

Various physical wound dressings have proven to be efficient in both covering and protecting wounds[10, 13] In this trial, we opted for a material that can secure the sponge in place, offering convenience in terms of ease of application and, time-saving benefits for both the patient and surgeon. Flowable composite material fulfilled all the requirements[29].

Since it had been applied as a stent in both groups, it was not considered as a variable. Palatal wounds could not be left without any physical dressing for obvious ethical reasons.

When analyzing the current study's results; regarding the gender and age of the participating sample, there were no differences concerning the measured parameters. This was expected due to the strict inclusion criteria in order to avoid any bias or confounding variables. The same with respect to the location of the grafted areas no significant difference was noted between the two groups.

Moreover, the thickness of the harvested grafts in our study; and consequently, the depth of the palatal wounds showed no significant difference between the two groups. This was intentional in an attempt to standardize all study variables as much as possible.

This study's aim was to assess the impact of melatonin on palatal mucosa wound healing after graft procurement through digital planimetry. Palatal wound healing was evaluated by measuring the difference in remaining wound area (in mm^2) using photo-digital planimetry. The percentages of remaining surgical wound areas were monitored and compared between the two groups.

After one week, regarding the remaining wound surface area, melatonin group showed a lower remaining wound surface area, although that was not statistically significant. This finding could be attributed to the action of melatonin on TGF-β. This growth factor is a crucial mediator in the trans-differentiation of fibroblasts into myofibroblasts. Myofibroblasts are crucial cells in wound healing by secondary intention; they contain actin and myosin, and act to contract the wound; decreasing the remaining wound surface area. Melatonin reduces the action of TGF-β and therefore reduces wound contraction at test site.

These outcomes are in accordance with Yeon Kim et al[38], who reported that melatonin hinders trans-differentiation of renal Interstitial fibroblasts to myofibroblasts by suppressing reactive oxygen species.

As regards healing index of Landry , results revealed no statistically significant difference between groups after one week. This could be due to the inherent subjectivity of this index, since it relies on visual assessments and subjective scoring of wound criteria. It's possible that small variations in the healing process may not be detected solely by the naked eye.

Regarding the VAS values there was no statistically significant difference between groups as well. The

possible reason could be linked to the application of the composite as a stent in both groups, since it provided a similar degree of physical protection of the wounds in both groups.

It might appear that melatonin had limited impact -regarding wound healing- on clinical level seen by naked eyes, yet on histological level, many trials praised the tremendous effect of this material on healed tissues[39]. Histological analysis allows visualization of newly formed tissue structure and precludes subjectivity.

Pugazhenthi et al[39], assessed the quality of scarring in full-thickness incisional wounds by histological analysis, the results showed that melatonin treatment improved the quality of the scar. Another histological study performed by Amin et al [40], who found that melatonin treated ulcers had better collagen bundle organization and additional angiogenesis.

Further research is required to establish -in greater detail- the effects of melatonin on the wound-healing process.

In brief, larger sample size, longer follow -up periods may help elucidate the effect of melatonin in palatal wound healing. Also, histological evaluation is needed to evaluate the true nature of healing after the use of gelatin loaded hydrogels.

CONCLUSION

Within the limitation of the present clinical research, we concluded that melatonin could be beneficial in improving palatal wound healing.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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