ACTA ODONTOLOGICA SCANDINAVICA, 2018
VOL. 76, NO. 3, 212–219
https://doi.org/10.1080/00016357.2017.1403648

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ORIGINAL ARTICLE

Topical simvastatin gel as a novel therapeutic modality for palatal donor site wound healing following free gingival graft procedure

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ABSTRACT

Objective: Autogenous soft-tissue grafting is a commonly used procedure nowadays in dentistry. However, the prolonged healing time needed for the donor site leads to increase the patient's pain and discomfort. Statin has been observed to be beneficial in reducing bacterial burden, improving epithelization and wound healing. The aim of this study was to evaluate intra-oral topical application of simvastatin/chitosan gel (10 mg/mL) over the palatal donor site following free gingival graft (FGG) procedure.

Material and methods: Subjects indicated for FGG procedure were divided into four groups. Group I: Simvastatin suspension (S), group II: simvastatin/chitosan gel (SC), group III: chitosan gel (C), group IV: petroleum gel (P). Treatment was applied three times/day for the following 7 days. Wound healing was evaluated at day 3, 7 and 14 post-surgery. A visual analogue scale (VAS) was used to measure the experienced discomfort at 1, 3, 5, 7 and 14 days.

Results: Statistical significant reduction in wound-healing scores was observed after 3 and 7 days for group II compared to other groups ($p = .015$). A significant reduction was also observed in VAS score for group II compared to other groups at day 1, 3, 5 and 7.

Conclusion: Topical application of S/C gel could be used as a novel therapeutic modality that improved healing and reduced pain in the palatal donor site following FGG procedure.

ARTICLE HISTORY
Received 16 June 2017
Revised 30 October 2017
Accepted 2 November 2017

KEYWORDS
Graft; healing; palate; simvastatin; wound; chitosan

Introduction

Autogenous soft-tissue grafting is a procedure that is used extensively nowadays in dentistry. Harvesting-free gingival graft (FGG) and subepithelial connective tissue graft (SCTG) have been the standard techniques used to increase soft-tissue volume [1,2]. Soft-tissue augmentation can be indicated in many conditions such as localized ridge defects, ridge preservation and as preprosthetic surgical technique [1–4]. Despite the anatomical and individual limitations that might hinder autogenous soft-tissue grafting, the common drawbacks of using autogenous tissue are generally due to the harvesting procedure and the prolonged healing time needed for the donor site which increases the patient's morbidity [5,6]. In addition, pain, discomfort and numbness are common patients complain following the surgery that may last for several weeks [7–9].

Intra oral wounds are mostly susceptible to oral bacterial insults that complicate the healing process. Bacterial contamination can induce inflammation, inhibit angiogenesis and secrete proteolytic enzymes leading to tissue damage thus, disturbing the wound healing [10]. The application of antimicrobial and antiseptic agents on wounds have been previously evaluated [11–12]. However, drawbacks such as limitation of systemic and topical antibiotics to promote wound healing as well as the development of drug-resistant microorganisms have been observed [10]. Moreover, topical antiseptics, such as povidone–iodine, chlorhexidine and hydrogen peroxide, have shown toxic effect on host cells that might hinder the healing process [13]. Therefore, obtaining new materials that provide better wound care through decreasing bacterial contamination and enhancing wound healing have been considered an essential demand [14].

Statins, the well-known lipid-lowering class of compounds have been proven to possess both anti-inflammatory and antibacterial actions [15,16]. They have been used in the management of wound healing, psoriasis, alopecia and other inflammatory conditions [10,17,18]. Statin has been observed to be beneficial in reducing bacterial burden, improving epithelization and wound healing as well as exerting a direct effect on pathogenic microorganisms and fungi [18,19].

Rego et al. [20] conducted the first animal study who reported a favourable effect of statin in wound healing. 0.2 mL of simvastatin microemulsion (10 mg/mL) was applied once daily until wounds healed in rats. They concluded that topical simvastatin have reduced wound inflammation and bacterial loads in contaminated skin wounds. Asia et al. [21] found that topical application of simvastatin promotes lymphangiogenesis and angiogenesis during wound healing in

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genetically diabetic mice. In their study, wound was covered with a semipermeable polyurethane dressing after topical application of simvastatin in petroleum jelly (a mixture of 5 mg of simvastatin and 995 mg of jelly). Ten milligrams of the mixture was applied to the wound on days 0, 4, 7, and 10 after creation of the wound.

Bitto et al. [19] observed that daily systemic administration of simvastatin (5 mg/kg) in diabetic mice enhances vascular endothelial growth factor (VEGF) production and improve skin wound healing. However, systemic administration at an extremely high dose was used to obtain angiogenic effects, and this is inapplicable for clinical use. Oral statins similar to other medications can cause adverse effects when used for prolonged time or high dose. Thus, topical application of statins with avoidance of systemic adverse effects may be useful for wound healing, in which prolonging statin effect while lowering the possible side effects [10,16].

Recently, local delivery of statins as an adjunctive therapy to SRP has been investigated. Statins are believed to increase expression of bone morphogenetic protein-2 gene and vascular endothelial growth factor, thus, stimulating bone regeneration [22–23]. Moreover, statins are supposed to decrease the level of tissue destruction in periodontal disease [24]. Two recent reviews [25,26] advocated that statins may also hold beneficial effects in periodontal immunomodulation and bone formation. Ambrósio et al. [24] observed in their systematic review a beneficial effect of local use of statins regarding periodontal regeneration. The local application of statins have been shown to be beneficial especially in patients with unfavourable regeneration potential [26].

Chitosan is a natural biodegradable biocompatible polymer that has been used for many biomedical applications. Over the last few years, many studies evaluated its application as drug delivery system, wound healing and antibacterial agent [27]. Its antimicrobial character is due to the presence of its cationic nature. Chitosan-dependent antimicrobial activity has been observed against various microorganisms, such as fungi and bacteria [28]. Chitosan has been used as drug carrier for hardly soluble drugs to increase their bioavailability. Chitosan is considered as a new innovative material, that is widely used as a drug carrier to reduce the rate of drug clearance [29].

The intra-oral topical application of statin might be a novel therapeutic modality to improve wound healing while exerting antibacterial action, simultaneously. The goal of our study was to evaluate the intra-oral topical application of simvastatin in chitosan gel (10 mg/mL) over the palatal donor site following free gingival graft procedure. To the best of our knowledge, this is the first clinical study performed to cover this point.

Material and methods

The subjects consisted of 40 patients with mean age range: 25–40 years referred to the Department of Periodontology, Faculty of Dentistry, Alexandria University, Egypt. The patient inclusion criteria were the presence of narrow zone of attached gingiva $\leq$1 mm on the facial aspect of the mandibular/maxillary anterior area, non-pregnant and non-smoker, no history of systemic medications intake within the past three months and no record of allergies and systemically healthy. The prerequisite of the donor site were; the presence of palatal mucosal thickness $\geq$4 mm measured by bone sounding using a periodontal probe [30], the absence of periapical or palatal pathologies and the absence of excessive forces (e.g. mechanical forces from orthodontics and traumatic occlusions). Each subject signed a detailed informed consent form, the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and ethical approval was granted by the Ethics Committee of Faculty of Dentistry, Alexandria University.

The subjects were divided randomly into four groups (10 patients each), following FGG-harvesting procedure, different coverage techniques were used to induce healing of the palatal wounds: group I: Simvastatin suspension (S), group II: simvastatin/chitosan gel (SC), group III: chitosan gel (C), group IV: petroleum gel (P).

In all subjects, pre-surgical preparation consisted of oral hygiene instructions and scaling and root planning. Four weeks later, a re-evaluation was performed and all patients recorded an O'Leary index $\leq$10% [31].

Materials

Chitosan gel (2.5%) was prepared by dispersing 125 mg chitosan flakes in 5 mL 1% acetic acid [32]. The mixture was left for about 1 h to allow polymer swelling and gel formation. Simvastatin gel was prepared by suspending 50 mg simvastatin in 1 mL 1% acetic acid, followed by mixing with 4 mL chitosan gel that was prepared as previously mentioned, so that the final concentration of simvastatin is 10 mg/mL [20] and of chitosan is 2.5%. Simvastatin suspension (10 mg/mL) was prepared by dispersing 50 mg of simvastatin in 5 mL of purified water. Petroleum gel was used as a negative control [21].

The surgical procedures

The recipient site was prepared similar to the technique described by Langer and Langer [33]. After adequate local anaesthesia was obtained (2% lidocaine with 1:100,000 epinephrine) a horizontal incision was made in the mucogingival junction with a #15 scalpel blade. A split-thickness incision was extended distally 1 to 2 teeth further than the planned graft area. The raised tissue was discarded, and a periosteal bed was prepared. Gauze moistened with saline was placed over the recipient bed until graft placement.

Donor site preparation

Breifly, after local anaesthesia administration, a partial thickness incision $\geq$2 mm [30] in thickness using #15 scalpel blade was performed to harvest the FGG. The bevelled part of the blade was used as a guide in order to standardize the wound depth. The outline of the graft was determined by using a template of the recipient site. In order to standardize the

surgical wound, the donor site was located between the first premolar and the first molar and 2–3 mm away from the gingival margins of the corresponding teeth (20 × 10 mm). Trimming of the graft was done if needed to adapt to the shape and size of the recipient site. The graft was then positioned and adapted to the recipient site. The coronal part of the graft was positioned at the MJG level, then sutures was taken to adapt the graft firmly in this position and stabilized with cross sling sutures (5-0 silk). A gauze soaked in saline was applied for 5 minutes over the graft with very mild compression to avoid the creation of any dead space.

Covering the donor site

After harvesting the free gingival graft from the palate, the donor site was irrigated with sterile saline, and haemostasis was achieved with gauze moistened in saline. Then, four different covering techniques were applied over the donor site to protect the surgical region. In group I: Simvastatin suspension (S), group II: simvastatin/chitosan gel (SC), group III: chitosan gel (C), group IV: petroleum gel (P). In group II, the donor site was covered with simvastatin/chitosan gel (10 mg/mL). The patient was instructed to apply 1 mL of the gel or the suspension three times/day for the following 7 days postoperatively. The gel/suspension was provided in 3-mL syringes and patients were instructed to apply it over the wound using cotton swap and to avoid drinking or eating for one hour after the gel application. A non-steroidal anti-inflammatory analgesic was prescribed for the patients (twice/day). The patients recorded their analgesic consumption following the surgical procedure.

Clinical evaluation

(A) Wound healing

The type of wound bed [34] was evaluated by clinical observation and the scores were determined as follows:

4 - Necrotic Tissue (Eschar): black, brown or tan tissue that adheres firmly to the wound bed

3 - Slough: yellow or white tissue that adheres to the wound bed

2 - Granulation tissue: pink or beefy red tissue with a shiny, moist, granular appearance.

1 - Epithelial tissue: for superficial ulcers, new pink or shiny tissue that grows in from the edges or as islands on the wound surface.

0 - Closed/Resurfaced: the wound is completely covered with epithelium.

Evaluations were obtained at day 3, 7 and 14. Comparison of total scores measured over time and the duration of healing in days provide an indication of the improvement or deterioration in wound healing. The wound-healing score was evaluated by two blinded examiners.

(B) visual analogue scale scores VAS [35]

Patients were given log diaries and instructed to record the intensity of pain and discomfort on the VAS, consisting of a scale from 0 to 100 (a 10 cm line). On this scale, 0 and 100 represented 'no pain or discomfort' and 'the worst pain or discomfort imaginable,' respectively. The recordings were made at day 1, 3, 5, 7 and 14 days, post-surgery (p.s).

(C) analgesic consumption

The patients recorded their analgesic consumption following the surgical procedure.

Statistical analysis

Sample size was calculated (https://select-statistics.co.uk/calculators/sample-size-calculator-two-proportions/) using the following assumptions: alpha error = 5%, study power = 80%, % closure without intervention after 7 days (control) = 40% [21,36] and % closure with simvastatin with chitosan =95%. The minimum required sample was calculated to be 8 which was increased to 10 to make up for losses due to no show ups at follow-up visits. The comparisons between different groups at several time points for wound healing were done using Kruskal–Wallis test and Mann–Whitney test. ANOVA and *post hoc* test were used for VAS and analgesic consumption. Significance was accepted at $p < .05$. Intra-examiner evaluation revealed minor differences between the two readings; all measurements were within 5% of the initial measurements and inter-examiner agreement was kappa <0.05.

Results

Forty patients divided randomly into four groups (10 patients each) were involved in this study. After harvesting FGG, different coverage techniques were performed to protect the palatal wounds (Figures 1–4).

(1) Healing score

A significant reduction in wound-healing score was observed for group II compared to other groups at 3 and 7 days p.s. However, at 14 days p.s., no significant difference was found between the different groups. Intra-group comparison revealed a significant reduction between 7 and 14 as well as between 3 and 14 day p.s in all groups. Significant reduction between 3 and 7 days was only observed in group II (Table 1).

(2) VAS score

A statistically significant reduction in VAS score for group II was observed compared to other groups at day 1, 3, 5 and 7 p.s. ($p = .001$, $p = .001$, $p = .003$ and $p = .003$); respectively. At 14 days, no significant difference was found between the studied groups ($p = .581$). (Figure 5)

(3) Analgesic consumption

Regarding the number of analgesic consumption days for group I were 5 days, 2 days for group II, 4 days for group III

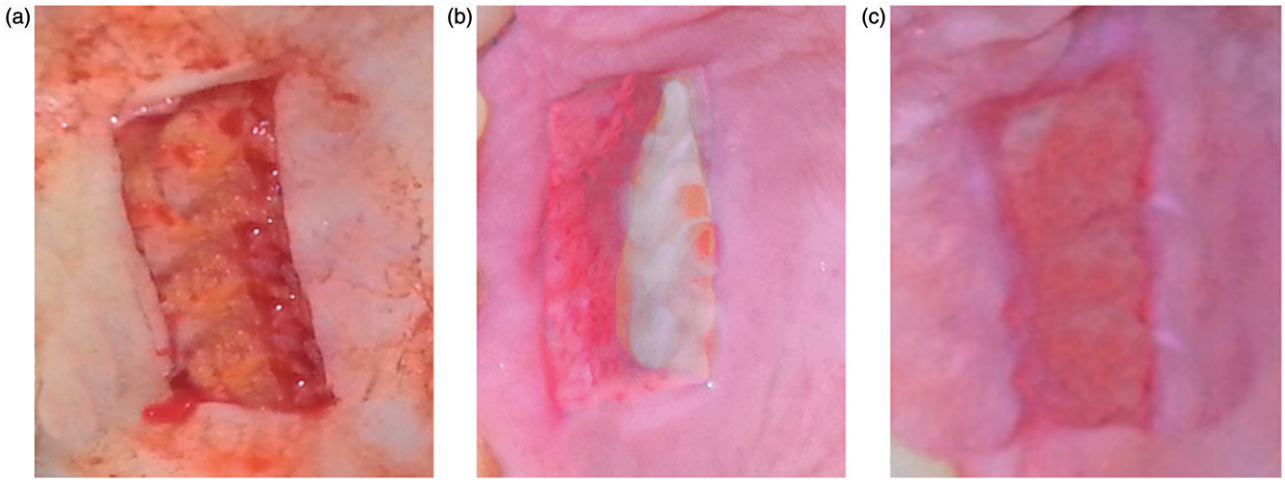

Figure 1. Clinical view of the palatal donor site for group I (simvastatin). Immediately after FGG procedure (a) after 3 days of drug application (b) and after 7 days (c).

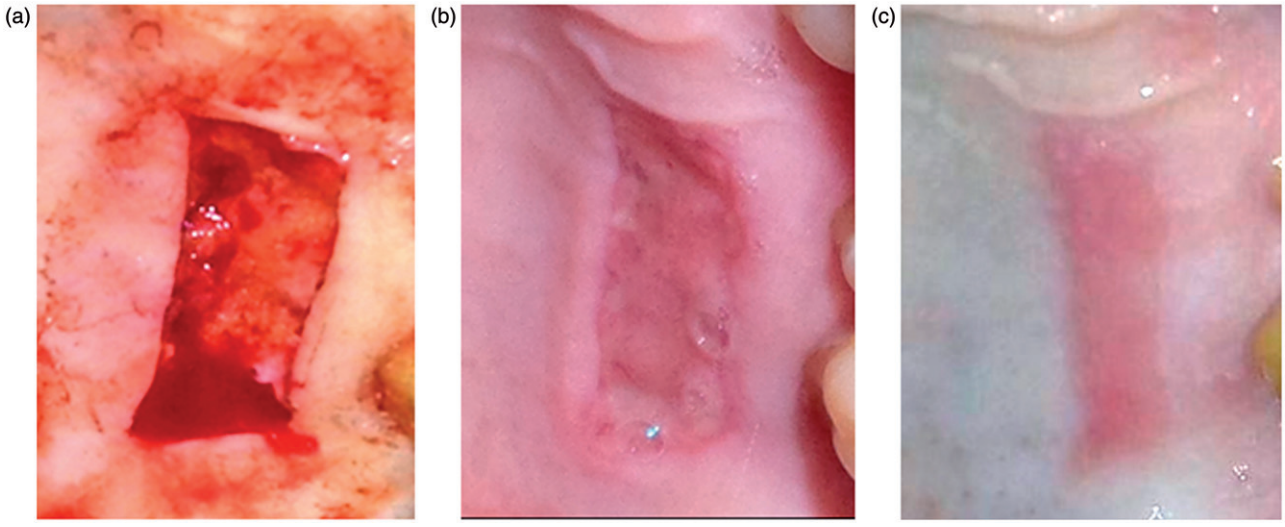

Figure 2. Clinical view of the palatal donor site for group II (simvastatin/chitosan). Immediately after FGG procedure (a) after 3 days of drug application (b) and after 7 days (c).

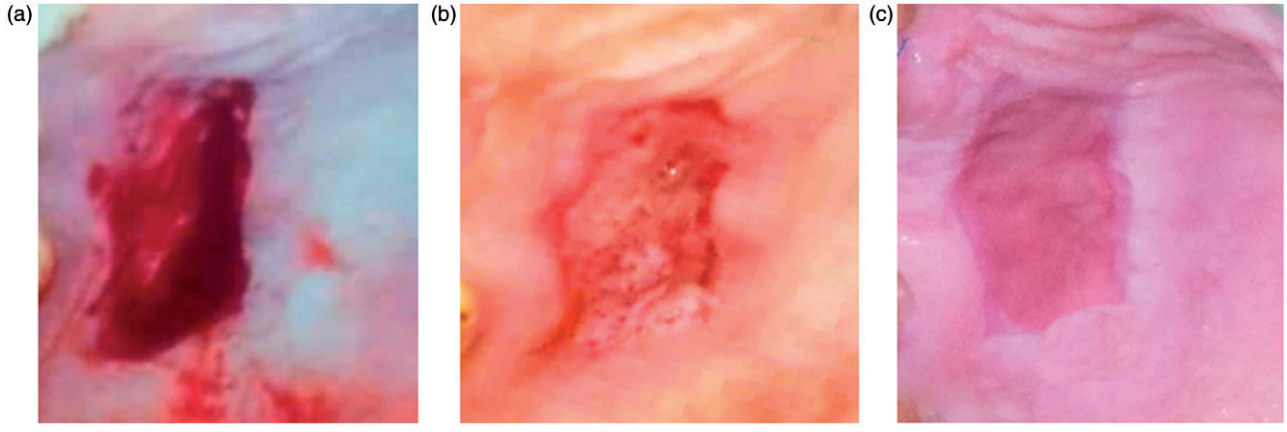

Figure 3. Clinical view of the palatal donor site for group III (chitosan). Immediately after FGG procedure (a) after 3 days of drug application (b) and after 7 days (c).

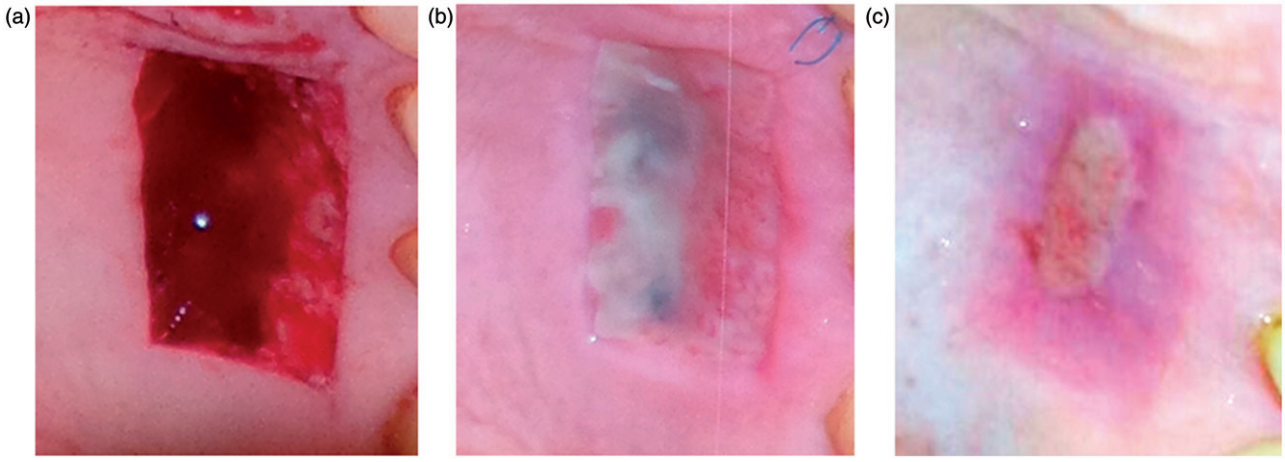

Figure 4. Clinical view of the palatal donor site for group IV (petroleum gel). Immediately after FGG procedure (a) after 3 days of drug application (b) and after 7 days (c).

Table 1. Comparison between groups I (S), II (SC), III (C) and IV (P) according to wound healing score.

Healing score	Day 3 Mean ± SD	Day 7 Mean ± SD	Day 14 Mean ± SD	p_4
Group I simva (S)	3.0 ± 0.0	2.78 ± 0.53	0.0 ± 0.0	.021[bc]*
Group II simva/chitosan (SC)	2.0 ± 0.0	1.43 ± 0.53	0.0 ± 0.00	.015[abc]*
Group III chitosan (C)	3.0 ± 0.0	2.51 ± 0.49	0.0 ± 0.00	.135[bc]*
Group IV petroleum gel (P)	3.0 ± 0.0	2.88 ± 0.45	0.54 ± 0.35	.031[bc]*
p_1	.030*	.010*	–	
p_2	.030*	.030*	–	
p_3	.030*	.010*	.954	

Qualitative data were analysed using Kruskal–Wallis test and Mann–Whitney test between different groups.
p_1: p value for comparing between group II (simva/chitosan) and group I (simva).
p_2: p value for comparing between group II (simva/chitosan) and group III (chitosan).
p_3: p value for comparing between group II (simva/chitosan) and group IV (petroleum gel).
p_4: p value for comparing between 3, 7 and 14 days post-operatively.
[a]Statistically significant between 3 and 7 days post-operatively.
[b]Statistically significant between 7 and 14 days post-operatively.
[c]Statistically significant between 3 and 14 days post-operatively.
*Statistically significant at $p \leq .05$.

and 7 days for group IV. A significant difference was observed between group II and IV ($p = .03$). (Figure 6)

Discussion

In this study, simvastatin/chitosan gel was used to cover the palatal donor site after FGG surgery in comparison to simvastatin or chitosan or petroleum gel. The results showed significant differences between groups in post-operative healing score during the early post-operative period as well as for pain VAS scores.

Due to the removal of the epithelial layer of the palatal mucosa, the FGG wound usually heals within 2–4 weeks with secondary intention [5]. There is evidence that complete epithelialization of the palatal donor site within 4 weeks after surgery is considered normal healing for FGG harvesting areas [37–39]. However, during this healing period, the palatal wound has been associated with a high incidence of pain at the donor site. In the current study, the presence of palatal mucosal thickness ≥4 mm was considered as essential inclusion criteria for donor site. Delayed wound was observed in case of leaving insufficient thickness of soft tissue (<2 mm) on the palatal bone after gingival graft harvesting [30,37]. Thus, in the current study, approximately 2 mm FGG was harvested from the palate to avoid insufficient residual tissue thickness on the palate that might prolong the healing time.

Wessel et al. [40] observed that the mean VAS pain scores at 3 days and 3 weeks postoperatively following FGG were 48 and 36, respectively. In our study, the mean VAS pain scores at 3 days were 45, 27, 40 and 50, respectively, for group I, II, III and IV. These values are smaller than those obtained by Wessel et al. [40] and Ersoz et al. [41]. This could be attributed to the potentiation of wound healing that simvastatin and chitosan exert.

Our VAS score results are in consistent with those observed in Kecili et al. [38] study. They evaluated the effectiveness of a medicinal plant extract (MPE) in achieving haemostasis and early wound healing at palatal donor site after free gingival graft (FGG) compared to applying wet gauze. They observed significantly lower VAS score values for (MPE) group than wet gauze group during the first post-operative week.

In general, wound healing can be divided into three stages, inflammation, tissue formation and tissue remodelling [2,42]. In the early phase of an open wound, the body's primary objective is to obtain a seal against outside to prevent the invasion of microorganisms. The rate of palatal wound epithelization is determined by the relationship between proliferative and migratory activity of the peripheral keratinocytes and collagen synthesis of the exposed connective tissue [43]. The acceleration of epithelization and wound closure rate can be explained through positive contributions of statin by inhibiting adhesion and extravasation of leukocytes into the inflammation sites, which in turn results in reduced co-stimulation of T cells and decrease of inflammatory cytokines. Thus, facilitating wound healing during the early stage. While stimulating macrophages infiltration [21]; which in turn stimulates proliferation of fibroblasts, keratinocytes and endothelial cells in the proliferative stage. Angiogenesis stimulation which in turn promotes

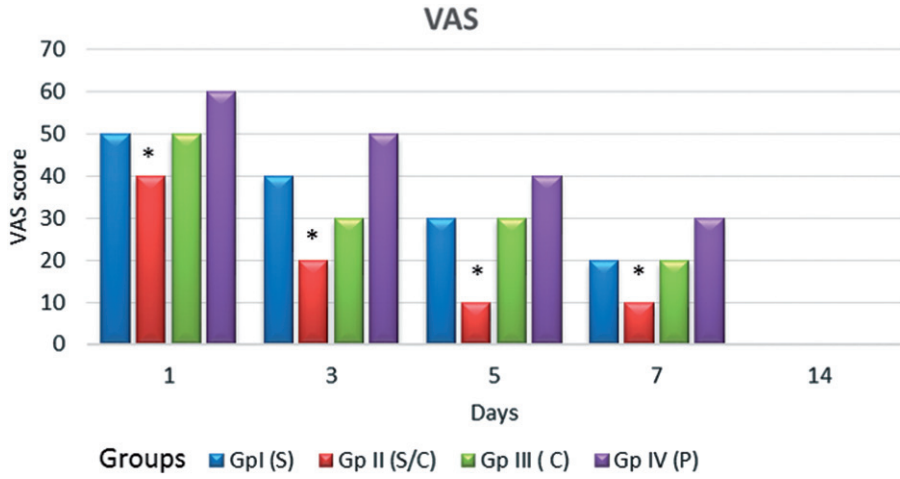

Figure 5. Visual analogue scale scores VAS for group I (S), group II(S/C), group III (C) and group IV (P) during the study period. (*significant difference, $p < .05$).

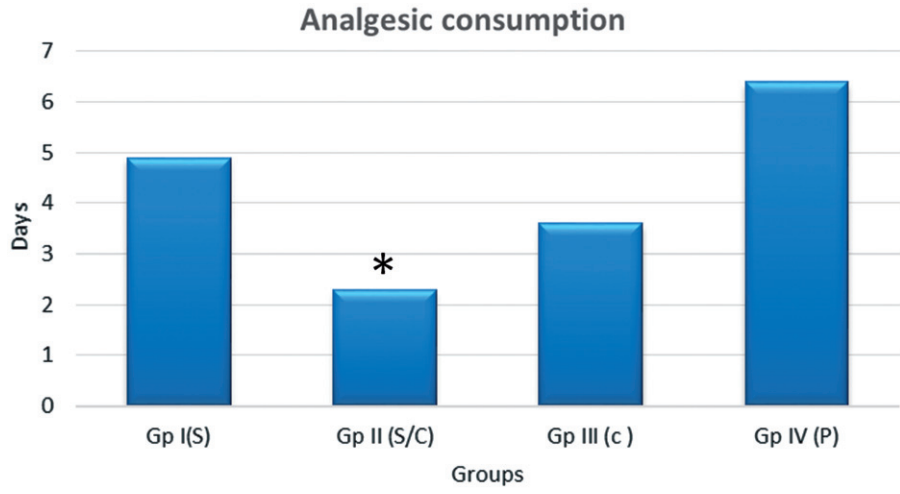

Figure 6. Analgesic consumption days for patients in the studied groups. A significant difference was observed between group II and IV ($p = .03$).

macrophage infiltration as well as inducing vascular endothelial growth factor (VEGF) production and stimulating re-epithelialization have been reported [21,44,45].

In our study, we evaluated the wound defect using score from 0 to 4. At 3 days p.s, the simva/chitosan group II showed score 2 that represents pink granulation tissue covering the defect. These findings are in agreement with those of Thoma et al. [2,46]. They evaluated clinically and histologically the palatal wound healing with or without a collagen matrix application. Significant differences were found between CM sites and control sites at 4 and 8 days. The histological examination revealed wound bed keratinization amounted to 18% (CM) and 12.4% (control) at day 4.

The better healing score observed for group II in comparison to other groups comes in agreement with previously reported results by Adami et al. [47] who showed that topical application of 1% simvastatin (equivalent to the concentration used in our study) ointment twice daily for five consecutive days didn't not cause skin barrier disruption on inflamed skin.

Chitosan is a biodegradable natural polymer with low toxicity and good biocompatibility. Due to its ability to form a thin film, it has been used as a drug carrier for several biomedical preparations [48–50]. Due to the washing effect of the saliva and the continuous friction that the palatal wound is subjected to, a bioadhesive carrier for the simvastatin would be essential to prevent the rapid washing of the drug from the wound area. This could explain the results observed for the simvastatin suspension group. In the present study, the simvastatin/chitosan gel showed better results than simvastatin suspension. This could be attributed to the bioadhesive property of the chitosan as a carrier that decreased the drug clearance rate thus, allowing prolonged contact time with the wound.

Soane et al. [51] reported that chitosan had reduced the rate of drug clearance from the nasal cavity, due to its bioadhesive property and increased its bioavailability. Furthermore, previous studies showed that chitosan had stimulatory effect on fibroblasts and inflammatory cells thus, it enhances granulation and organization during wound healing [52,53]. Consequently, chitosan gel had been used as wound dressing due to its biocompatible, biodegradable, haemostatic, anti-infective and wound-healing property [28,54]. Thus, the combined use of chitosan and simvastatin had a synergistic effect on accelerating the wound-healing process.

As a limitation, although extreme care was taken to obtain the graft in constant thickness, the technique used in the present study might have impaired the standardization of wound depth, compared to other relevant studies [39,46]. Possible alternative approaches that could be done in future studies to avoid such a limitation is by using a metal template or mucotome. Moreover, absence of quantitative wound dimension measurements, colour matching and wound sensitivity measurements can be rendered as another limitation.

To sum up, this study supports that topical application of simvastatin at such a lower concentration (10 mg/mL) is considered a safe and promising novel modality for promoting wound healing. Together with its antibacterial activity and inflammation modulation such wound care strategy could potentially minimize the risk of bacterial infection during wound healing. Further clinical trials are warranted to validate the findings of our study on larger sample size and infected wound model.

To the best of our knowledge; this was the first study designed to evaluate the clinical effect of topical simvastatin gel applied to cover the donor site after FGG procedure.

Conclusion

We can conclude that topical simvastatin/chitosan gel (10 mg/mL) can be used as a novel therapeutic modality that improves healing and reduces pain in the palatal donor site following FGG procedure.

Disclosure statement

No potential conflict of interest was reported by the authors.

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