THE EFFECT OF A CALENDULA BASED TOPICAL FORMULA VERSUS OXIDIZED REGENERATED CELLULOSE ON PALATAL WOUND HEALING: A RANDOMIZED CONTROLLED CLINICAL TRIAL

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ABSTRACT

INTRODUCTION: Free palatal and sub-epithelial connective tissue grafts are the most commonly used grafts in periodontal plastic surgeries. The surgical technique of free palatal grafting requires harvesting of soft tissue from the palatal donor site and transplantation to an intraoral recipient one. Post-surgical complications and atypical healing processes have been described in the literature. In order to reduce the morbidity associated with mucogingival surgery, there are several ways to manage the donor site after soft tissue harvesting. Various topically applied formulas and hemostatic agents have been employed to manage post-operative healing at the donor site.

OBJECTIVES: to assess the effects of Calendula based topical formula on palatal wound healing after free palatal graft surgery in comparison to oxidized regenerated cellulose.

MATERIAL AND METHODS: This randomized, controlled trial included twenty-four surgical sites for palatal graft procurement, divided equally into two groups, managed by the topical application of either a Calendula based formula (Group-I) or oxidized regenerated cellulose (Group-II). Palatal wound healing was assessed using photo-digital planimetry on the day of the surgery and; at seven and fourteen days, post-surgical. Pain was assessed by visual analogue scale (VAS) one, four and seven days post-surgical.

RESULTS: The VAS score results showed no significant difference at days one, four and seven when comparing group I and II. For the percentages of remaining wound area, there was no significant difference in the percentages of remaining wound area. However, at day seven, there was a significant decrease in the percentage of remaining wound area in group II when compared to group I. Comparing the percentages of remaining wound area at day seven and fourteen in group I, there was a significant decrease of the percentages of remaining wound area at day fourteen. The same was found in group II. For group I, there was no significant difference between any of the recording periods. However, for group II, there was a significant decrease in the VAS scores between day one and seven, and also between day four and seven.

CONCLUSION: Both materials were beneficial in improving palatal wound healing. However, oxidized regenerated cellulose exhibited better pain reduction following palatal graft procedures as reported by the study subjects when compared to the topical Calendula group

KEY WORDS: Calendula, Chlorhexidine Gluconate, Connective Tissue, Hemostatics, Oxidized Cellulose, Pain, Panthenol, Tissue Harvesting, Visual Analog Scale, Wound Healing

RUNNING TITLE: Effect of a Calendula Based Topical Versus Oxidized Regenerated Cellulose on Palatal Wound Healing.

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INTRODUCTION
Free palatal grafts (FPG) and subepithelial connective tissue grafts (SCT) have been found to be the most commonly used grafts in periodontal plastic surgeries. They are used to correct mucogingival defects in morphology, amount and/or location of soft tissue and underlying bone support for teeth and dental implants (1, 2).

Several hemostatic agents have been utilized to manage post-operative bleeding at the donor site. Currently the interest is usually focused upon topically applied natural therapies (3-5).

Oxidized regenerated cellulose is manufactured in a woven fabric of varying thickness which is often preferred for topical application. The mechanism of action that causes oxidized regenerated cellulose to accelerate clotting is not completely understood but appears to be a physical effect rather than an alteration of the physiologic clotting mechanism. The product absorbs in the body over seven to fourteen days and has shown to be bactericidal in vitro against gram-negative and gram-positive organisms (6).

Calendula officinalis - an annual plant of the family Asteraceae, blossoms from May to October. Its flowers are utilized in medicinal formulations. It is native to Central Europe and the Mediterranean and flourishes naturally at sunny places all over North America and Europe. The extract produced from this plant has been broadly utilized in Europe since the twelfth century as a topically applied anti-inflammatory agent (7). Apart from its anti-inflammatory property, Calendula reduces the permeability of capillary walls and accelerates the healing of wounds (8). When the Calendula brew was applied to tissues, favorable morphological changes in the cell structure could be observed. These were displayed by several and extended projections and an escalated number of cells. This fact was confirmed by Fronza et al. indicating that Calendula officinalis extracts stimulated the proliferation and migration of fibroblasts at low concentrations (9). The aim of this study was to assess the effect of a Calendula based topical formula in comparison to with oxidized regenerated cellulose on palatal wound healing after free palatal graft surgery.

MATERIAL PATIENTS
A total of twenty-four surgical sites from free palatal graft procedures were included in the study. Patients were recruited from the patients’ pool of outpatient clinic at the Department of Oral Medicine, Periodontology, Oral Diagnosis and Oral Radiology, Faculty of Dentistry, Alexandria University. The purpose and nature of the study were explained to the patients and an informed consent was obtained from patients who agreed to participate in this trial prior to any procedure. Treatment was in accordance to the principles of the modified Helsinki code for human clinical studies (2013) (10).

The clinical study was conducted following the ethical guidelines for conduct of research on human subjects, by the Faculty of Dentistry, Alexandria University (IRB NO:00010556 - IORG 0008839).

SAMPLE SIZE CALCULATION
A sample size of 12 sites per group (number of groups = 2) (total sample size = 24 sites) was the enough required sample as statistically significant with 80% power and at a significance level of 95% (accepted α error = 0.05). Sample size per group did not need to be increased to control for attrition bias (11). The sample size was calculated using G Power version 3.1.9.2 (12).

SAMPLES GROUPING
Inter- and intra-examiner reliability
Inter- and intra-examiner reliability when tracing the remaining wound area was calculated by intraclass correlation coefficient (ICC) (13).

OXIDIZED REGENERATED CELLULOSE
• Soft, lightweight, layered absorbable hemostat made from oxidized regenerated cellulose. It is supplied as staple fiber in envelopes of the (1in x 2in) (Code No. 1961) (Surgicel® FibrillarTM absorbable hemostat, Ethicon, Johnson & Johnson Medical Devices Companies, US, LLC).

CALEDULA BASED TOPICAL FORMULA
• Calendula extract (extracted from crude freshly collected Calendula flowers (Emtenan Herbal Shop, Alexandria, Egypt))
• Chlorohexidine HCl (Alexandria Pharmaceuticals, Alexandria Egypt).
• D- Panthenol (Shaanxi Bolin Biotechnology Co., Ltd. China).
• Carboxymethyl cellulose sodium (CMC-Na) (Prolabo Pharmaceutical Chemicals Co, Cairo, Egypt).
• Gelatin (Gel) (ADWIC El-Nasr Pharmaceutical Chemicals Co, Cairo, Egypt).
• Pectin (Pect, industrial grade, with about 70% methyl esterification) (Alamerya Pharmaceuticals, Alexandria, Egypt).
• Polycarbophil (PL) (Noveon® AA1 a sample gift, Lubrizol, Belgium).

METHODS

CALENDULA BASED TOPICAL FORMULA PREPARATION

PREPARATION OF MUCOADHESIVE GEL FORMULATION:
Mucoadhesive gel formulations were prepared based on the original orabase formulation first introduced by Extra Pharmacopoeia (14). The modified orabase gel was prepared by mixing equal ratios of modified orabase base (8% Carboxymethylcellulose sodium (CMC Na): 8% Pectin (Pect): 8% Gelatin (Gel)) and different concentrations of
polycarbophil, PL (4.5& 6%). Modified oral adhesive base was prepared by first dispersing the calculated amount of Gel in hot water. After cooling, CMC-Na and Pectin were added gradually, with continuous stirring (15, 16). Polycarbophil base was prepared by dispersing the calculated amount of PL in double distilled water and allowed to completely gel under continuous stirring. The different formulations were assembled by adding different ratios of the modified oral adhesive base and different concentration of PL (1:1(4.5%), 1:1(6%), 0:1, and 1:0) All placebo formulations were evaluated by a palatability study to choose the most acceptable formulation with respect to taste and odor. The chosen concentration based on the palatability study was oral adhesive base: PL 4.5% in a 1:1 ratio.

PATIENTS SELECTION
Patients requiring free palatal graft procedures were included in this study.

INCLUSION CRITERIA
1. Patients had at least one site of natural dentition or dental implants that needed to be treated by free palatal graft surgery for indications including: progressive recession, planned prosthodontics, presence of a mucogingival deformity, or a lack of keratinized gingiva.
2. Patients’ age ranged from 20 to 40 years.
3. Patients had at least 4 mm thickness of palatal mucosa (at donor site).

EXCLUSION CRITERIA
4. History of smoking.
5. Patients that had any known disease that interfered with periodontal surgery.
6. Patients that had any dermal or autoimmune diseases.
7. Patients that had any previous adverse reactions to the products (or similar products) used in this study.
8. Pregnant and lactating women.
9. Patients that had a palatal infection.

The same previously discussed procedure was adopted for the preparation of the Calendula loaded base with the addition of the required amount of 1% Calendula extract (extracted from crude freshly collected Calendula officinalis flowers in the laboratories of the faculty of Pharmacy and drug Manufacturing, Pharos University in Alexandria) or 1% Chlorhexidine HCl with 5%D- Panthenol in the final mixing step.

GROUPING
The selected twenty-four sites were randomly assigned to the following groups:
- Group I: Twelve sites, where the donor site was dressed with the Calendula based topical formula.
- Group II: Twelve sites, where the donor site was dressed with Surgicel® FibrillarTM absorbable hemostat Oxidized Regenerated Cellulose wound dressing.

RANDOMIZATION
Surgical sites that complied with the inclusion criteria were randomly assigned using a computer-generated list of random numbers to one of the two groups (https://www.randomizer.org/). The allocation was performed by a trial independent individual and the allocation ratio was intended to be equal (17).

PRE-SURGICAL PREPARATION
All the patients underwent phase I therapy to establish optimal plaque control and gingival health conditions. The patients were instructed to perform plaque control measures and then re-assessed at 4 weeks after the initial therapy. Only those with a full mouth O’Leary plaque index score ≤15% (18) and a full mouth gingival index score (19) of zero were enrolled in the surgical procedure (20).

SURGICAL PROCEDURES
Free palatal graft surgical procedure of either natural teeth or dental implants was performed. The procedure of obtaining an autogenous free palatal graft was performed based on the principles of the classical approach as described by Sullivan and Atkins in the late 1960’s (21, 22).

The width and the length of the graft were measured by a standard UNC-15 periodontal probe (Hu-Friedy Mfg. Co., LLC, Chicago, Illinois, United States) to the nearest millimeter (Figure 1). The probe was also placed perpendicular to the harvested graft, to measure its thickness (23). After the free palatal grafts were harvested from the palatal donor sites, an adrenaline soaked sterile surgical gauze was applied to the palatal wounds with pressure maintained for 5 minutes to control the bleeding.

Following that, for group I, the Calendula based topical formula was applied to cover the donor sites (Figure 2a-2c). For group II; Surgicel® FibrillarTM Absorbable hemostat Oxidized Regenerated Cellulose dressing material was trimmed to the size of the wound and applied to the palatal donor site, following the manufacturer’s instructions. Cross horizontal sling sutures were done to stabilize the dressing material after hemostasis was achieved (Figure 2d-2f).

POST-SURGICAL 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 licence of Abbott Laboratories – Italy) (600 mg when needed) All patients were instructed to rinse with 0.12% chlorhexidine gluconate mouth rinse (DG Care, AL ESRAA PHARMA. Egypt) twice per day for 2 weeks starting 24 hours after the surgical procedure.
Patients from group I were further instructed to apply the topical formula on the palatal donor sites 3 times per day; once after each meal for one week. They were instructed to wash their hands with soap and water before each application to avoid any possible contamination of the wound and were asked to apply the gel with care to avoid any injury to the donor site that would potentially retard the healing process. In patients from group II, the sutures were removed from the palatal donor sites after 1 week. The patients were recalled after 1, 4, 7 and 14 days for postoperative follow-ups. Clinical follow-up photographs was taken during each follow-up visit (24).

**Figure 1:** Surgical procedures, a. Measuring palatal thickness, probe perpendicular, more than or equal to 4mm, b. Graft template on palate, c. Graft outline traced by blade tip, d. Measuring harvested graft thickness using UNC-15 probe

**Figure 2:** post-surgical procedures, a. Group I donor site with scale, b. Calendula based topical formula, the yellow tube contained the loaded Calendula extract with oral adhesive gel, and the white tube contains loaded gel of 1% Chlorhexidine HCl with 5%D- Panthenol, c. Group I donor site with Calendula based topical formula applied, d. Group II donor site with scale (Surgicel® FibrillarTM Absorbable Hemostat), e. Surgicel® FibrillarTM Absorbable Hemostat,f. Group II donor site with Surgicel® FibrillarTM Absorbable Hemostat sutured in place.

**PATIENTS ASSESSMENT**

**SUBJECTIVE ASSESSMENT (VISUAL ANALOGUE SCALE)**

Visual Analogue Scale [VAS] is a simple measurement tool that measures the intensity of pain as recorded by the patient. It ranges across a continuum from none to an extreme amount of pain in a straight horizontal 10 cm line [from 0-100 mm] (25). One day after the surgery, the patients were asked to grade overall severity of their symptoms regarding the palatal donor site on the VAS then it was reassessed after 4 and 7 days after surgery using the same scale. The patients' scores were recorded in millimeters (26).

**OBJECTIVE ASSESSMENT**

**PHOTO-DIGITAL PLANIMETRY (DIFFERENCE IN REMAINING WOUND AREA [MM2])**

Standardized clinical photographs of the palatal surgical sites were taken on the day of the surgery and on 7 and 14 days after the surgical procedure. Attention was paid to capture the wound area photographs in a perpendicular way. A standard-sized visual scale was placed next to the wounds as a reference for wound size measurements and analysis. Seventy-two photos in total, taken from twenty-four sites at three different time points, were used for the final analysis. The analysis was performed by two different examiners and the grouping of the sites was not revealed to eliminate any bias. The pictures were imported into a computer software (ImageJ 1.49v) and wound areas were calculated using computerized planimetry.

**DATA MANAGEMENT AND STATISTICAL ANALYSIS**

The data was processed and analyzed using Statistical Package for Social Sciences program SPSS (20.0) software (Armonk, NY: IBM Corp, USA). The study included descriptive and analytical data. A P-value of less than 0.05 was considered statistically significant.

**RESULTS**

**DEMOGRAPHIC DATA AND PALATAL GRAFT DIMENSIONS**

The demographic data are presented in Table 1. For group I, 3 males and 9 females participated in the current study with a mean age of 27.58 ± 7.27 years. For group II, 2 males and 10 females participated in the current study with a mean age of 32.75 ± 8.49 years. Statistical analysis comparing group I and II revealed no significant difference between the 2 groups regarding gender and age of the participants. The palatal graft dimensions are presented in Table 2. Statistical analysis, comparing group I and II, revealed no significant difference between the two groups regarding the dimensions of the grafts used.

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**VAS SCORES**
The VAS score results are presented in Figure 3. The VAS scores, recorded at day one, were 18.3 ± 14.0 and 23.3 ± 16.1 for group I and group II, respectively. At day four, they were 30.0 ± 22.2 and 16.7 ± 9.8 for group I and group II, respectively. Finally, at day seven, the scores were 20.8 ± 20.2 and 7.5 ± 10.6 for group I and group II, respectively.

Comparing group I and group II, there was no significant difference at day one, day four and day seven. For group I, there was no significant difference between any of the recording periods. However, for group II, there was a significant decrease in the VAS scores between day 1 and day 7, and also between day 4 and day 7.

**PERCENTAGE OF REMAINING WOUND AREA**
The percentages of remaining wound area are presented in Figure 4. The percentages of remaining wound area at day 7, were 95.40 % ± 38.93 and 64.80 % ± 20.77 for group I and II, respectively. At day 14, they were 29.62 % ± 25.91 and 18.38 % ± 10.42 for group I and II, respectively. Comparing groups I and II at day 14, there was no significant difference in the percentages of remaining wound area. However, at day 7, there was significant decrease in the percentages of remaining wound area of group II compared to group I.

Comparing the percentages of remaining wound area at day 7 and day 14 in group I, there was a significant decrease of the percentages of remaining wound area at day 14. The same was found in group II.

**Table 1:** Comparison between the two studied groups according to demographic data.

|                | Group I (n = 12) | Group II (n = 12) | Test of Sig. | P  |
|----------------|------------------|-------------------|--------------|----|
| Gender         |                  |                   |              |    |
| Male           | 3 (25.0)         | 2 (16.7)          | $\chi^2 = 1.282$ | 0.258 |
| Female         | 9 (75.0)         | 10 (83.3)         |              |    |
| Age            |                  |                   |              |    |
| Min. – Max.    | 21.0 – 40.0      | 21.0 – 49.0       | t = 1.602    | 0.123 |
| Mean ± SD.     | 27.58 ± 7.27     | 32.75 ± 8.49      |              |    |
| Median (IQR)   | 23.0             | 33.0              |              |    |

$\chi^2$: Chi square test  
t: Student t-test  
FE: Fisher Exact

**Table 2:** Comparison between the two studied groups according to graft dimensions

| Graft   | Group I (n = 12) | Group II (n = 12) | T   | P   |
|---------|------------------|-------------------|-----|-----|
| Width   |                  |                   |     |     |
| (mm)    | Min. – Max.      | 4.0 – 8.0         | 5.0 | 1.773 | 0.090 |
|         | Mean ± SD.       | 5.42 ± 1.08       | 6.25 ± 2.22 |    |
|         | Median           | 5.0               | 6.0 |     |
| Length  |                  |                   |     |     |
| (mm)    | Min. – Max.      | 7.0 – 16.0        | 9.0 | 1.260 | 0.221 |
|         | Mean ± SD.       | 10.58 ± 2.68      | 11.92 ± 2.50 |    |
|         | Median           | 10.50             | 11.0 |     |
| Thickness|                  |                   |     |     |
| (mm)    | Min. – Max.      | 1.50 – 2.0        | 1.50 | 1.685 | 0.106 |
|         | Mean ± SD.       | 1.63 ± 0.23       | 1.63 ± 0.23 |    |
|         | Median           | 1.50              | 2.0  |    |

t: Student t-test  
p: p value for association between different categories
DISCUSSION

In order to reduce the morbidity associated with mucogingival surgery, there are several ways to manage the donor site after soft tissue harvesting. The use of analgesics has become a standard of care after surgery to manage post-operative pain at the palatal donor site. In the current study, Ibuprofen was the drug of choice to manage post-operative pain. Ibuprofen works by inhibiting the cyclooxygenase (COX) enzymes, which convert arachidonic acid to prostaglandin H2 (PGH2). PGH2, in turn, is converted by other enzymes to several other prostaglandins (which are mediators of pain, inflammation, and fever) and to thromboxane A2 (which stimulates platelet aggregation, leading to the formation of blood clots). After oral administration, peak serum concentration is reached after 1–2 hours and up to 99% of the drug is bound to plasma proteins (27).

Adjuncts such as surgical dressings, collagen matrices, different suturing methods and plastic palatal protective stents have been used to improve patient comfort and reduce bleeding of donor sites (28). Several dressing materials have been described in the literature to enable successfully protecting the palatal wound without additional coverage of a palatal stent (29, 30). Rossmann and Rees (6) concluded that the use of hemostatic agents for the management of palatal wounds is the treatment of choice when performing free soft tissue grafts. Surgicel® fibrillarTM absorbable hemostat was used in this study as the material of choice in the control group for management of the palatal donor sites. Due to the positive effects of Calendula officinalis on the promotion of wound healing, epithelial growth and topical anti-inflammatory and antibiotic effects; it was chosen to be tested in this study to evaluate its effects on palatal wound healing following free palatal graft harvesting from the palate. However, it is a weaker inhibitor of adherence of microorganisms than CHX (31).

Therefore, a topical formulation of Calendula officinalis mixed with 1% Chlorhexidine and 5% D-Panthenol was prepared in an oral adhesive base to be tested, utilizing the positive effects of all three materials on wound healing and promoting wound epithelialization. The prepared topical formulation was used in the current study to evaluate its effects on palatal wound healing following free palatal graft harvesting from the palate. Panthenol is a biologically active stable alcohololic analog of pantothenic acid, a member of the B complex vitamins (vitamin B5) (32). Pantothenic acid is optically active, only the dextrorotatory isomer has biologic activity. Dexpanthenol (d-panthenol) is freely soluble in water and alcohol, practically insoluble in fats, and it is the most stable form of pantothenic acid in liquids. Dexpanthenol is used topically as an ointment, emulsion, or solution, at concentrations of 2 to 5%, as an adjunct in the treatment of various skin and mucosal lesions. Topical d-panthenol acts like a moisturizer, improving stratum corneum hydration, reducing trans-epidermal water loss and maintaining skin softness and elasticity. Activation of fibroblast proliferation, which is of relevance in wound healing, has been observed both in vitro and in vivo with d-panthenol (33).

When analyzing the results of the current study; regarding the gender and age of the participating sample, there were no effects on the measured parameters. Similarly, it has been reported that demographic factors (age and sex) had no effect on the healing process of mucosal wounds, except in extremes of age (34).

The thickness of harvested grafts in the current study; and consequently, the depth of the palatal wounds created, was 1.5-2 mm. This was in accordance with the documented literature which reported that the optimal graft thickness was found to be 2 mm for one-step or for a direct approach and 1-1.5 mm for an indirect or a two-step procedures (35).

The aim of the present study was to assess the effects of a Calendula based topical formula in comparison to Surgicel® fibrillarTM as dressing materials on: palatal wound healing after free palatal graft surgery using photo-digital planimetry within two weeks as well as pain after harvesting free palatal grafts from palatal donor sites utilizing Visual Analogue Scale (VAS) within seven days. The impact of both materials on wound healing was assessed without a palatal stent as a bias factor that could interfere with the outcomes.

In the current study, palatal wound healing was assessed by measuring the difference in remaining wound area (mm2) using photo-digital planimetry. The percentage of the remaining surgical wound areas were monitored and compared between the two groups. At day seven, there was a statistically significant decrease in the percentages of remaining wound area in the Surgicel® fibrillarTM group compared to the Calendula group demonstrating faster healing in the Surgicel® fibrillarTM group after one week post-operatively. However, comparing both groups at day fourteen postoperatively, there was no significant difference in the percentages of remaining wound area. Moreover, when comparing the percentages of remaining wound area at day seven and day fourteen in the Calendula group, there was a significant decrease of the percentages of remaining wound area at day fourteen. The same was found for the Surgicel® fibrillarTM group. However, the difference in the mean percentage of remaining wound area between day seven and day fourteen was greater in the Calendula group (65.78%) in comparison to the Surgicel® fibrillarTM group (46.42%), demonstrating faster healing in the Calendula group between seven days and fourteen days post-operatively.

For the patients in the Calendula group, there were many factors that could affect the results of the research that were left quite literally in the hands of the patients, namely their adherence to the protocol of applying the topical formula. A few challenges were faced; the topical formula was supplied to the patients in two separate tubes, one tube containing the loaded Calendula extract and the other containing the loaded chlorhexidine and d-panthenol with the patient mixing both...
together immediately before applying to the palatal wound. The Calendula was kept in a separate tube to avoid any unknown interferences with either the Chlorhexidine and/or d-Panthenol during storage that could potentially affect its normal mechanism of action. The topical formulation was also free of any chemical additives commonly used to extend the shelf life of topical formulas.

On the other hand, the patients in the Surgicel® fibrillarTM group did not have to do anything with regard to the palatal wound healing process apart from adhere to standard post-surgical instructions. In accordance, only one of the palatal wounds from the Surgicel® fibrillarTM group exhibited an increase in wound area size during the first week of healing. The aggregate effect from the previously explained factors serves as a possible explanation to the demonstrated faster healing in the Surgicel® fibrillarTM group after one week post-operatively when compared to the Calendula group.

As previously stated, the palatal wounds demonstrated faster healing in the Calendula group between seven days and fourteen days post-operatively. Unlike Surgicel® fibrillarTM which is only a hemostatic dressing and physical barrier, Calendula has been proven to exhibit biologically active properties such as: an increase in the proliferation and migration of human fibroblasts and keratinocytes grown in cultures, increased angiogenesis seen in the chorioallantois membrane model and decreased collagenase activity (36). The biologic effect that Calendula has in promoting wound healing and speeding wound epithelialization supports the evidence from the current study that demonstrated faster healing in the Calendula group between seven days and fourteen days, postoperatively. This result is also attributed to the effects of Chlorhexidine and d-Panthenol also present in the topical formula. The former was proven to exhibit antimicrobial properties, whereas the latter (37), exhibits moisturizing and anti-inflammatory properties and fibroblasts activation in the case of d-Panthenol (33). These properties altogether improved the wound healing potential of the tested sample.

The resorption rate of Surgicel® fibrillarTM and has been described in the literature. According to previous reports, Surgicel® fibrillarTM resorbed in 7-10 days (38). In the current study, all of the patients in the Surgicel® fibrillarTM group reported that the dressing material dislodged during the first week post-operatively. Upon clinical examination and sutures removal at post-operative day seven, none of the wounds showed evidence of the remaining Surgicel® fibrillarTM dressing material. Therefore, the resorption rate of the Surgicel® fibrillarTM used in the current study could not be assessed.

Several methods have been used to study wound healing and epithelialization in the previous literature, such as direct clinical inspection, examination of clinical photographs, utilization of hydrogen peroxide solution and staining agent. Previously, Thoma et al. described the use of photographs in evaluation of human palatal wounds and the images were imported into computer software for wound area measurement (39). In the current study, examination of clinical photographs was the chosen method used to assess healing. Additionally, to enhance accuracy of wound size measurement, the concept of photo-digital planimetry was employed.

In this study, the VAS pain scores were examined at postoperative days one, four and seven. The mean VAS score on day one for the Calendula group was lower than the Surgicel® fibrillarTM group. The patients in the Surgicel® fibrillarTM group had sutures in place to stabilize the dressing material on the palatal wound. The extra trauma from the palatal sutures could explain the higher mean VAS scores for the Surgicel® fibrillarTM group on day one. Also, Surgicel® fibrillarTM has a physical hemostatic effect, it swells in contact with blood, adheres to the wound edges and vessels forming an artificial coagulum rather than an altering the physiologic clotting mechanism. Extra tension on the cross horizontal sling sutures stabilizing the Surgicel® fibrillarTM group could have resulted from this swelling effect, potentially also explaining further the higher mean VAS scores for the Surgicel® fibrillarTM group on day one in comparison to the Calendula group. Yet, the mean VAS scores of the Calendula group were higher than the Surgicel® fibrillarTM group on days four and seven. This can be explained by the fact that the Surgicel® fibrillarTM was acting as a physical barrier protecting the palatal wounds from mechanical, chemical and thermal irritation. On the other hand, the topical Calendula based formula offered little, if any, physical protection to the palatal wounds once applied.

However, in comparing VAS scores of the Calendula and Surgicel® fibrillarTM, there was no significant difference at day one, day four and day seven. Pain is an inherently subjective multifactorial experience and has proven to be complex to assess, evaluate, and manage (40). In this study, the VAS pain scores reported by different patients had a large variety within the same group, larger samples might be able to give more reliable results.

CONCLUSIONS

Referring to the limitations of the present study, we conclude that the Eosinophilic count is a strong indicator to determine the stage and degree of differentiation of the tumors.

Within the limitations of the present study, we conclude the following:

- The use of photo-digital planimetry proved to be a very useful tool for the objective assessment of wound healing by measuring the remaining wound area.
- Regarding pain control; the barrier action of the Surgicel® fibrillarTM group aided in reducing post-operative pain reported by the test subjects during the initial week of healing in comparison to the topical Calendula gel group.

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• Regarding palatal wound healing; both materials were beneficial in improving palatal wound healing, with the topical Calendula gel group exhibiting improved wound healing potential during the second post-operative week. However, using either material would be beneficial for palatal wound healing and a choice of either could be dictated by patient co-operation, material cost and initial dimensions of the palatal wound.

STATEMENT OF CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

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