

Effect of hypochlorous acid spray on healing of intraoral open wounds: A pilot clinical study

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Abstract

Background: The use of a free mucosal graft is a highly reliable technique for augmenting the width of keratinized gingiva. Furthermore, this technique is employed for the purpose of managing marginal tissue recession and enhancing vestibular depth. The Free Mucosal Graft procedure results in an exposed wound site as the epithelial layer is removed from the donor location. The wound typically takes two to four weeks to heal through secondary wound healing. Nevertheless, the most frequently reported complications include bleeding from the location where tissue was taken, the exposing of bone after surgery, and pain experienced after the operation. The primary aim of this study is to assess the impact and potential advantages of using 0.003% Hypochlorous acid (HOCl) topically vs using mouthwash with 0.12% Chlorhexidine (CHX) on the healing process of palatal donor sites throughout the postoperative period.

Methods: Randomised clinical trial was conducted on ten patients who underwent free mucosal grafts. The patients were categorised into two groups: a test group (n=05) and a control group (n=05). Postoperative wound healing was assessed using Landry’s wound healing index, pain/discomfort was measured using a visual analogue scale, and the number of days needed for complete re-epithelialization was determined using the hydrogen peroxide bubble test. These assessments were conducted on the 1st, 3rd, 7th, 14th, 21st, and 28th day. The results were statistically compared using both Anova and chi-square tests.

Results: Significant statistical differences were seen between two groups in terms of wound healing, postoperative pain/discomfort, and the number of days required for re-epithelialization. The test group exhibited improved wound healing and reduced pain intensity as measured by the Visual Analogue Scale (VAS).The test group patients took less days to complete Re

epithelialisation. The treatment tolerability was acceptable and comparable in both groups.

Conclusion: The findings of this study demonstrated that the use of a topical hypochlorous acid spray at a concentration of 0.003% for a duration of 14 days resulted in significant improvements in postoperative discomfort, wound healing, and the rate at which the palatal donor sites re-epithelialized.

Keywords: Plastic Periodontal Surgery, Postoperative Complications, Surgical Procedures, Wound Healing.

Summary

As a result of obtaining free gingival grafts from palatal tissue for mucogingival surgical procedures, secondary wound healing of the donor area occurs. This wound in the palatal region can cause discomfort and pain every time patients use their mouths. The use of Hypochlorous acid spray can reduce postoperative complications by accelerating wound healing, Re- epithelialisation and reducing pain.

Introduction

Insufficient keratinized gingiva in individuals increases the susceptibility of the gingival margin to inflammation and marginal tissue recession. Palatal free mucosal grafts are frequently utilised for gingival augmentation and are widely regarded as the most reliable and popular procedure for extending the width of keratinized tissue.

In addition, this technique can address cosmetic concerns, improve the depth of the vestibule, prevent future development of gum recession, and contribute to the stability of the gum line [1]. The removal of the epithelial layer in the palatal region results in an open wound site when using a free mucosal graft. This wound site typically heals through secondary intention within a period of two to four weeks [2,3]. However, there may be certain problems at the place where the donor tissue is

taken from the palate. The often reported consequences encompass intense postoperative pain, postoperative bone exposure, and exostoses [4-6]. Despite the utilisation of several materials such as platelet-rich fibrin (PRF), herbal extract, collagen matrices, and chemotherapeutic drugs in the donor area, there is no definitive agreement on their effectiveness in preventing these problems [7,8,9].

Hypochlorous acid (HOCl) exhibits potent antibacterial, antibiofilm, and anti-inflammatory capabilities, effectively targeting a wide spectrum of microorganisms [10, 11]. HOCl, a naturally occurring molecule produced by neutrophils to eliminate infections, is utilised in both dermatological and cosmetic operations due to its limited microorganism resistance, safety, and antibacterial efficacy [12]. Chlorhexidine digluconate (CHX) is widely recognised as the most effective antiplaque agent and is currently regarded the best option available [13]. Nevertheless, the limited acceptance of the medication by patients can be attributed to its adverse effects.

The bacterial bioburden in the tissue has been difficult to decrease, as evidenced by findings. When it comes to lowering the number of germs that are present in a chronic granulating wound, antibiotics that are administered systemically are useless. As a consequence of this, the techniques that have proven to be most effective have been transitory biologic dressings or topical antibiotics. The use of topical antibiotics that are effective when administered systemically for causes other than wound infection is not suggested. This is due to the increased risk of allergic reactions or the possibility of drug resistance in bacteria. It has been demonstrated that non-antibiotic antimicrobials and antiseptics, such as mafenide acetate cream, silver sulfadiazine, and povidone-iodine, are cytotoxic to the cellular components that are involved in the process of wound healing. Stabilised hypochlorous

acid, which is produced by adding sodium hypochlorite to a sodium chloride solution in sterile water, followed by the addition of a hydrochloric acid solution, and finally maintaining the mixture at a pH ranging from 3.5 to 5, has been demonstrated to have beneficial antibacterial and anti-inflammatory properties in vitro. The fact that it is necessary to maintain its narrow pH range in the therapeutic wound setting can be considered a disadvantage.

To the best of our knowledge, there has been no research conducted on the impact of HOCl and CHX on the healing of palatal wounds following a free mucosal transplant. The objective of this study was to evaluate and compare the impact of topically administered HOCl and CHX on wound healing, postoperative pain/discomfort, and the duration of complete re-epithelialization of the donor site.

Materials and methods

A randomised controlled clinical study was conducted on a sample of 10 patients. Subjects were assigned to two groups, Group A (Test) and Group B (Control), using a random allocation method. Group A received a topical spray of HOCL (0.003%) twice daily for 14 days, along with a 15 ml rinse of Chlorhexidine (0.12%) for 30 seconds, also twice daily for 14 days. Group B received only the Chlorhexidine rinse. The allocation of subjects to the groups was determined by flipping a coin, after obtaining their informed consent. The Full-mouth plaque score (FMPS) and full- mouth percentage bleeding score (FMBS) were determined by assigning a binary score to each tooth surface (1 for presence of plaque/bleeding, 0 for absence) and calculating the percentage of total tooth surfaces that exhibited plaque/bleeding, as detected using a periodontal probe. The recruitment period for this study extended from July 1st to September 30th, 2023. The

study included individuals who needed a free mucosal graft and met the following criteria: they were in good overall health with an ASA I physical status, aged between 18 and 60 years, and had a bleeding and plaque score of less than 10%. Participants were ineligible for the trial if they were currently smoking, had coagulation issues, or were unwilling to utilise additional hypochlorous acid and chlorhexidine. Each surgery was performed by a single periodontist utilising a consistent surgical approach to minimise variances in technique. A superficial cut was made in the area between the back corner of the canine tooth and the front corner of the first molar tooth, ensuring it was at least 2 mm away from the gum line. This was done after numbing the roof of the mouth with a local injection of 2% lidocaine with 1:100,000 epinephrine.

A mucosal graft measuring 15 x 8 mm and with a thickness of approximately 1-1.5 mm was harvested from the specified location using a scalpel number 15C. The graft's thickness was assessed at its midpoint using an endodontic reamer and a calliper on a smooth surface. The adipose and glandular tissue were excised from the graft, and subsequently, the graft was contoured to conform to the recipient site.

The patients were administered a 500 mg dose of paracetamol, an analgesic medication. The patients in the experimental groups, excluding the control group, received HOCl oral spray to be administered to the wound sites on the roof of the mouth twice a day for duration of 14 days. To avoid disrupting the stabilisation of the clot established in the surgical area, all topically applied treatments were initiated 6-8 hours following the procedure. Patients were instructed to abstain from eating, drinking, or rinsing their mouths for around 30 minutes after the spray was applied.

They were also urged to promptly report any negative effects that occurred. Patients were advised to refrain from vigorous scrubbing or causing any injury to the operative area for a period of 3 weeks. Every patient was closely observed, and a periodontist collected measurements on days 1, 3, 7, 14, 21, and 28.

The main patient-reported outcome measure (PROM) in this study was to assess the healing status of the palate wound using the Landry Wound Healing Index (WHI 1985). Visual evaluation of the donor site was conducted on the 3rd, 7th, 14th, 21st, and 28th days after the surgery. The index evaluates tissue colour, palpation reactivity, presence of granulation tissue, epithelialization of incision edges, and degree of suppuration on a scale of 1 (very poor) to 5 (excellent) [14]. The secondary PROM assessed patient's subjective experience of pain and discomfort during chewing following the extraction of a free mucosal graft. This assessment was done using a numerical rating scale (VAS) ranging from 0 (indicating no pain) to 10 (representing the most severe pain). The evaluations were conducted on the 1st, 3rd, 7th, 14th, and 21st days [15]. The palatal region's epithelialization was documented based on the application of 3% hydrogen peroxide to the area and the presence or absence of effervescence. Clinical assessment of complete epithelization (CE) involved observing the surface characteristics and clarity of the wound contour. The results were documented as either "yes" or "no" [16].

Results

The acquired data underwent statistical analysis using SPSS software. An intergroup comparative assessment was conducted to evaluate the mean Landry Wound Healing Index score based on duration and groups. This assessment utilised statistical methods including Repeated Measures ANOVA and Independent t-test

(Table 1, Fig 1). The results indicated that the HOCL+CHX group demonstrated superior wound healing on the 3rd and 7th day, with statistically significant findings.

However, there was not a substantial disparity observed after the seventh day. An intergroup comparative assessment of the mean Visual Analogue score based on duration was conducted using repeated measures ANOVA and Independent t test (Table 2, Fig 2). The results revealed that the HOCL+CHX group exhibited a superior VAS score, and this difference was statistically significant on the 1st and 3rd day. A comparative assessment of complete re-epithelialization between different groups was conducted using the Cochran's Q test and Chi square test (Table 3, Fig 3). The results showed that all participants in the HOCL+CHX group achieved complete re- epithelialization by the 21st day, whereas only two participants in the CHX alone group achieved the same outcome. This difference was found to be statistically significant.

Table 1: Comparative assessment of mean Landry Wound Healing Index score according to duration and groups.

| Groups | Duration (Mean ± SD) | | | | p-value |
|----------------------------------|----------------------|-------------|-------------|-------------|---------|
| | Day 3 | Day 7 | Day 14 | Day 21 | |
| Hypochlorous acid+ Chlorhexidene | 2.00 ± 0.00 | 3.20 ± 0.44 | 4.80 ± 0.44 | 5.00 ± 0.00 | 0.000* |
| Chlorhexidene | 1.60 ± 0.54 | 2.80 ± 0.44 | 4.80 ± 0.44 | 5.00 ± 0.00 | 0.002* |
| p-value | 0.141 | 0.195 | 1.000 | - | |

Test applied: Repeated measures ANOVA, Independent t test



Fig. 1 Mean WHI values of groups

Inter group Comparative assessment of mean Visual Analogue score according to duration was done using Repeated measures ANOVA, Independent t test (Table 2, Fig 2) and it was found that HOCL+CHX group showed better VAS score and results were statistically significant for 1 st and 3 rd day

Table 2: Comparative assessment of mean Visual Analogue score according to duration and groups

| Groups | Duration (Mean ± SD) | | | | | p-value |
|----------------------------------|----------------------|-------------|-------------|-------------|-------------|---------|
| | Day 1 | Day 3 | Day 7 | Day 14 | Day 21 | |
| Hypochlorous acid+ Chlorhexidine | 3.40 ± 1.34 | 2.00 ± 1.00 | 0.20 ± 0.44 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.000 * |
| Chlorhexidine | 6.00 ± 0.71 | 3.60 ± 0.54 | 0.80 ± 0.44 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.000 * |
| p-value | 0.005* | 0.014* | 0.067 | - | - | |

Test applied: Repeated measures ANOVA, Independent t test

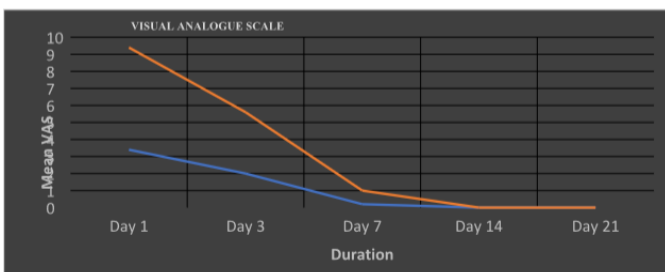


Fig. 2: Mean VAS pain, chewing, and burning values of groups

Inter group Comparative assessment of complete re-epithelialization according to duration was done using Test applied: Cochran’s Q test, Chi square test (Table3, Fig 3) and results stated that all participants of HOCL+CHX group had completed Re- epithelialization on 21 st day but only two participants of CHX alone group had completed Re-epithelialization and it was statistically significant.

Table 3: Comparative assessment of complete re-epithelialization according to duration and groups

| Groups | Duration n (%) | | | | | p-value |
|-----------------------------------|----------------|-------|--------|---------|---------|---------|
| | Day 3 | Day 7 | Day 14 | Day 21 | Day 28 | |
| Hypochlorous acid + Chlorhexidine | 0 | 0 | 0 | 5 (100) | 5 (100) | 0.000 * |
| Chlorhexidine | 0 | 0 | 0 | 2 (40) | 5 (100) | 0.000 * |
| p-value | - | - | - | 0.038* | - | |

Test applied: Cochran’s Q test, Chi square test



Fig. 3: In situ images of groups on the surgery, 3rd, 7th, 14th, 21st, and 28th days.

Discussion

Palatal donor site morbidity is the most adverse consequence following free mucosal graft surgery for patients. Several clinical investigations have prioritized improving the healing process of wounds on the roof of the mouth and minimising discomfort experienced by patients. Nevertheless, there is a lack of research in the existing literature that examines the impact of HOCl on the process of palatal wound healing following periodontal reconstructive surgery. The current study aimed to examine the therapeutic benefits of HOCL on the secondary wound healing of the palatal region and the patient’s discomfort following the free mucosal transplant operation. The main result of the study (WHI) showed that the group treated with HOCL+ CHX had the highest healing scores and reported the least pain, as measured by the Visual Analogue Scale (VAS), for up to 7 days after the surgery.

Furthermore, the group treated with HOCL+ CHX had significantly greater rates of CE on the 21st day compared to the group treated with CHX alone. Several essential processes are involved in the process of wound healing, including cell differentiation, proliferation, migration, and collagen deposition [17]. Due to its ability to render the SARS-CoV-2 virus inactive, HOCL has gained popularity in dentistry as a disinfectant and antiseptic within the COVID-19 epidemic [18]. During the activation of neutrophils, the respiratory bursts naturally generate peroxide (H₂O₂).

In the presence of Cl⁻ and H⁺, the activated granule enzyme myeloperoxidase transforms H₂O₂ to HOCl [19]. The HOCl produced plays a role in the bactericidal activity of neutrophils and is believed to be responsible for tissue damage in inflamed areas. HOCl exhibits antibacterial, antibiofilm, anti-inflammatory, and immunomodulatory characteristics. It also diminishes the activities of histamine, leukotriene B₄, IL-6, and IL-2 [20]. Super-oxidized solutions have been proposed to function as an inhibitor that stabilises the mast cell membrane, reducing the release of granules from mast cells caused by the cross-linking of IgE-antigen receptors. The lower VAS scores seen in the HOCl-administered groups compared to the other group can be attributed to the suppression of inflammation caused by the effects of HOCl, an antibacterial agent, on cytokine production.

Conclusion

Following free mucosal graft surgeries, HOCL may be the treatment of choice for the control of morbidity at the donor site in the palatal region. The use of hydrochloric acid resulted in a reduction in discomfort when compared to the control group, as well as an acceleration of wound healing and the observation of early epithelialization. As a result, this study demonstrated that the application of

0.003% topical hypochlorous acid spray for a period of fourteen days had a beneficial effect on postoperative pain, wound healing, and the early rate of re-epithelialization of palatal donor sites. However, longitudinal studies are required to provide evidence for this fact, and additional studies are required to be conducted in order to standardise the dose and frequency protocol of HOCL prescription.

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