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RESEARCH

# Comparison of hyaluronic acid, hypochlorous acid, and flurbiprofen on postoperative morbidity in palatal donor area: a randomized controlled clinical trial

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## Abstract

**Objective** This study aims to evaluate the effects of topical hyaluronic acid (HA), hypochlorous acid (HOCl), and flurbiprofen on postoperative morbidity of palatal donor sites after free gingival graft (FGG) surgery.

**Materials and methods** Sixty patients requiring FGG were randomly assigned into four groups: control, HA gel (600 mg/100 g high molecular weight hyaluronic acid), HOCl spray (170–200 ppm, ph7.1), flurbiprofen spray (0.075gr flurbiprofen). Topical agents were applied for 14 days, according to groups. Patients were followed for 28 days. Palatal healing was assessed with the Laundry wound healing index (WHI). Complete epithelization (CE) was evaluated with photographs and $H_2O_2$ bubbling. Pain, burning sensation, chewing efficacy, and tissue color match (CM) were evaluated using a visual analog scale (VAS). Postoperative analgesic consumption and delayed bleeding (DB) were also recorded.

**Results** HA provided better WHI values on the 7$^{th}$, 14$^{th}$, and 21$^{st}$ days compared to the other groups, respectively ($p < 0.05$). CE was formed on the 21$^{st}$ day in the HA group but on the 28$^{th}$ day in the other groups. HOCl and flurbiprofen groups were not different from the control group or each other in terms of WHI. HOCl had the lowest VAS scores of all time periods. DB was not observed in any group. Significantly fewer analgesics were taken in the topical agent-applied groups compared to the control group**.**

**Conclusions** HA exhibits a positive impact on the epithelization of palatal wound healing and color matching. HOCl and flurbiprofen provided less pain; however, they might have negative effects on palatal wound healing.

**Clinical relevance** 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 HA can reduce postoperative complications by accelerating wound healing and reducing pain. The topical use of flurbiprofen and HOCl can reduce patients' pain.

**Keywords** Operative · Pain · Plastic periodontal surgery · Postoperative complications · Surgical procedures · Wound healing

## Introduction

Free grafts from the palate have been used most commonly for gingival augmentation. It has been stated in the literature that free gingival graft (FGG) is one of the most reliable and frequently used methods to increase the amount of keratinized gingiva. In addition, this technique prevents and treats the progression of gingival recession, can eliminate aesthetic problems, and increases vestibular depth [1]. FGG leaves an open wound site due to the removal of the epithelial layer in the palatal region and heals in two to four weeks with secondary wound healing [2, 3]. However, some complications may occur at the recipient site. Excessive

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bleeding, postoperative bone exposure, bone exostoses, case of mucocele, severe postoperative pain, and recurrent herpetic lesions are the most documented complications [4–6]. Even if various materials with mechanical protection [7], platelet-rich fibrin (PRF) [8], herbal extract [5], and chemotherapeutic agents [9] are used in the donor area to prevent postoperative complications, there is no definite consensus.

Flurbiprofen, 2(3-fluoro-4-phenyl–phenyl)-propionic acid, is a non-steroidal anti-inflammatory drug (NSAID) that has been shown to be safe and effective in relieving postoperative pain [10, 11]. Flurbiprofen in the form of an oral spray can be found commercially and simply applied to the palatal region directly. It has been found to inhibit the increase of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, and interleukin (IL)-1β [12, 13], also reducing IL-6 expression [14] and inhibiting cyclooxygenase-2 (COX-2) expression [15].

Hypochlorous acid (HOCl) exhibits potent antimicrobial activities against a wide variety of microorganisms [16, 17]. HOCl, a naturally occurring molecule produced by neutrophils to destroy pathogens [18], is used in cosmetic and medical dermatological procedures due to its lack of microbe resistance, safety, and antimicrobial activity [17].

Hyaluronic acid (HA) is a natural polymer of glycosaminoglycans (GAGs) found in the joint synovial fluid and the extracellular matrix of the skin [19, 20]. It is naturally secreted during the proliferative phase of wound healing to stimulate migration and mitosis of fibroblasts and epithelial cells and has been shown to reduce levels of inflammatory mediators [21, 22]. It has been reported that HA has a positive effect on the healing of chronic wound ulcers of various etiologies, burns, and epithelial surgical wounds, regardless of the form in which it is administered topically (i.e., pad, cream, substrate) [23]. As a therapeutic agent, HA is used in tissue reconstruction [24], to accelerate wound healing [25, 26], in degenerative/inflammatory joint diseases, and in synovial fluid replacement [27, 28].

To the best of our knowledge, no studies have examined the effects of HOCl use on palatal wound healing after FGG surgery. The aim of this study was to compare the effects of locally applied HOCl, flurbiprofen, and HA on postoperative patient discomfort and wound healing of the palatal donor area in terms of pain, burning sensation, epithelialization, and color match after FGG surgery.

## Materials and methods

### Study pattern

This study was designed as a prospective randomized controlled clinical trial with parallel groups. The population of the study consists of all patients who were referred to FGG for gingival augmentation between March 5, 2019, and March 15, 2022, at Pamukkale University Faculty of Dentistry Department of Periodontology. Procedures were explained to the participants, and all of them signed consent forms before participating in the study. The study was approved by Pamukkale University Ethical Committee (05.03.2019/05) and submitted to ClinicalTrials.gov (NCT05386667). All procedures performed in this study comply with the ethical standards of the institutional and/or national research committees and the Declaration of Helsinki and its subsequent amendments or comparable ethical standards.

#### Inclusion criteria

- Patients with systematic conditions are classified as ASA Class I [29].
- Patients > 18 years requiring FGG surgery with ≤ 1 mm width of attached gingiva in the mandibular anterior and premolar region
- *Exclusion Criteria*
- Pregnancy, lactation, and taking contraceptive pills
- Oversensitivity or anaphylactic reactions that contraindicate the intervention
- Orofacial neurological symptoms
- Infections at operation zone
- Psychotropic medicine, sedative, or NSAI use can alter the sense of pain
- Pathological mental conditions (dementia, psychosis) and lack of cooperation
- Patients who refuse to sign consent forms
- Excessive gag reflex

Participants were randomly assigned to one of the 4 groups following a simple software-generated random number procedure via the "List Randomizer" application (https://www.random.org.lists). The power analysis of the study was carried out using the G*Power software program (G*Power v.3.1.9.2, Heinrich Heine University, Dusseldorf, Germany) accepting wound healing as the primary outcome variable. According to the power analysis of a previous study, the number of patients calculated for each group was 6, with $\alpha = 0.05$, $1\text{-}\beta = 0.95$, and $f = 2.386$ [7]. However, 15 patients for each group were included in the study, taking into account the loss of follow-ups (Fig. 1). All the patients received the initial periodontal therapy including scaling and root planning (SRP). Oral hygiene motivation was given to the patients, and they were recalled one month later. Full-mouth plaque score (FMPS) and full-mouth percentage bleeding score (FMBS) were recorded by assigning a binary score to each surface (1 for present, 0 for absent) and calculating the percentage of total tooth surfaces that

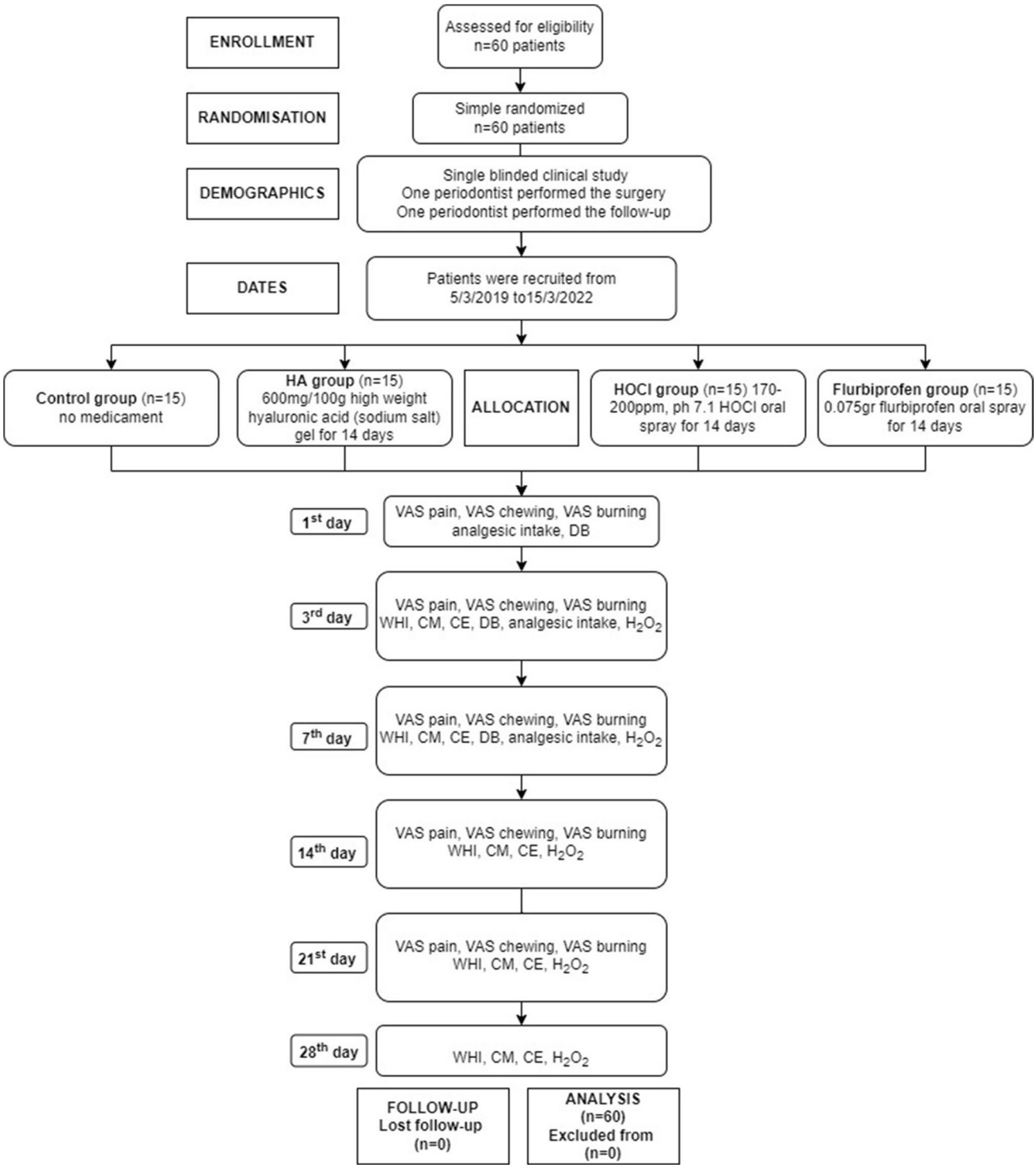


**Fig. 1** Flow diagram generated in accordance with CONSORT 2010 guidelines

revealed the presence of plaque/bleeding detected by the use of a periodontal probe (Hu-Friedy, Chicago, IL, USA) [30]. The number of teeth of the patients was also recorded.

### Study groups

– Control group: No topical agent was applied to the palatal area where the FGG was harvested.

- Hyaluronic acid (HA) group: 600 mg/100 g high molecular weight hyaluronic acid (sodium salt) gel was applied twice daily for 14 days to the palatal area where the FGG was harvested.
- Hypochlorous acid (HOCl) group: 170–200 ppm, ph. 7.1 HOCl oral spray was applied twice daily for 14 days to the palatal area where the FGG was harvested.
- Flurbiprofen group: 0.075gr flurbiprofen and additives (sorbitol, saccharin sodium, glycerin, polyoxyl 40 hydrogenated castor oil, methyl paraben, propyl paraben, ethyl alcohol, patent blue E131 (blue), menthol, sodium hydroxide) included, the oral spray was applied twice daily for 14 days to the palatal area where the FGG was harvested.

## Surgical intervention

In order to minimize the differences in surgical technique, all surgical procedures were performed with the same surgical technique by a single periodontist (ALA). The surgical application was applied by following the steps below briefly. After the palatal region was numbed with local infiltration anesthesia (2% lidocaine with 1:100,000 epinephrine), the area between the distal line angle of the canine and the mesial line angle of the first molar was marked as ≥ 2 mm away from the gingival margin, and a half-thickness incision was made. A FGG with a thickness of ≈1–1.5 mm and dimensions of 10 × 5 mm was obtained from the marked area with a scalpel no: 15C. The thickness was measured in the middle of the graft with an endodontic reamer and a caliper over a flat surface. The fat and glandular tissue were removed from the graft, and then the graft was shaped to adapt to the recipient site.

## Postoperative care

The patients were prescribed an analgesic drug (500 mg of paracetamol). The patients in the study groups other than controls were given HA gel, HOCl, and flurbiprofen oral spray to be applied to the palatal wound areas. Patients who were received HOCl and flurbiprofen oral spray instructed to take one dose (three sprays) to the donor site twice a day for 14 days. HA gel was prescribed twice a day for 14 days to apply with its special applicator to the palatal wound area. In order not to disturb the stabilization of the clot formed in the operation area, all locally applied products were started 6–8 h after the operation. Patients were given instructions not to eat, drink, or rinse for about 30 min after spray/gel application and advised to report the outcome if any adverse events occurred. Patients were instructed to avoid any hard brushing or trauma to the surgical site for 3 weeks. All patients were monitored, and measurements were taken by one periodontist (GTC) on days 1, 3, 7, 14, 21, and 28. Patients were asked if they needed to use analgesics and to note the amount they used in the 7-day postoperative period.

## Evaluated parameters

The primary outcome of the study was to measure the palatal wound healing status using the Landry Wound Healing Index (WHI) [31], which grades the wound healing on a scale of the 3rd, 7th, 14th, 21st, and 28th days postoperatively. This index, which has a score range of 1 (very poor) to 5 (excellent), evaluates tissue color, response to palpation, presence of granulation tissue, epithelialization of incision margins, and amount of suppuration.

The secondary outcomes of the study were to measure patients' perception of pain, discomfort while chewing, and burning sensation after FGG harvesting using a numerical rating scale (VAS) from 0 (no pain, no discomfort while chewing, no burning sense) to 10 (the worst pain imaginable, extreme discomfort while chewing, extreme burning sense) [32] on the 1st, 3rd, 7th, 14th, and 21st days, and the amount of analgesic consumption on the 1st, 3rd, and 7th days postoperatively. Tissue color match (CM) was assessed with adjacent and contralateral palatal tissue (0—no color matching to 10—excellent color matching) [32].

The patients were asked to keep a record of whether there was any bleeding in the palatal region, and at the same time, the presence of postoperative bleeding, also known as delayed bleeding (DB), was recorded as present (+) or absent (−) on the days when the patients came to the follow-up appointments.

The epithelialization of the palatal region was recorded according to the application of 3% hydrogen peroxide to the region and whether there was foaming or not, and was calculated as a percentage ($H_2O_2$ bubbling) [33]. Complete epithelization (CE) was also evaluated clinically by monitoring the surface characteristics and clarity of the wound contour and recorded as "yes" or "no" [26].

## Statistical analysis

All data were evaluated with SPSS 21.0 (SPSS Inc., Chicago, IL). Shapiro–Wilk test was used to test the data's normality. Since the data distributions were not normal, non-parametric tests were used to evaluate the analysis. The Chi-square test was used for demographic data (age, gender), CE, and DB. The number of teeth of the participants in all groups were compared with using one-way ANOVA post hoc Tukey test. Kruskal–Wallis post-hoc Mann–Whitney *U* with Bonferroni correction was applied to analyze the data of different groups (FMPS, FMBS, VAS pain, chewing, burning, WHI, CM, $H_2O_2$ bubbling, and analgesic consumption). The Friedman test was applied to evaluate the repeated measures within the groups. Data were expressed

as min–max (median), mean ± standard deviation (SD), and frequency. $p < 0.05$ was considered statistically significant.

## Results

A total of 60 patients took part in the study. The long duration of this study was due to the precautions taken during the COVID-19 pandemic. There was no drop-out among the 60 patients. All patients included in the study were followed for 28 days. Demographic data for the groups are presented in Table 1. FMPS, FMBS, and the number of teeth of all patients included in the study were similar between groups ($p > 0.05$). The patients participating in the study did not differ in terms of age and gender ($p > 0.05$). None of the patients developed adverse reactions to the agents used.

When our primary outcome, WHI, was examined, the HA group was found to be better in terms of wound healing scores compared to the HOCl group on the 3rd day ($p = 0.019$). The use of HA showed statistically better results in terms of wound healing compared to the control, flurbiprofen, and, HOCl groups on the 7th, 14th, and 21st days, respectively (Table 2, Fig. 2).

When the VAS data, which evaluated the postoperative pain levels of the patients, were analyzed, the pain levels of the patients in the HOCl group were found to be significantly lower than in the flurbiprofen group ($p = 0.007$) and the control groups ($p = 0.033$) on the 1st day. On the 3rd day, patients in the HOCl group felt significantly less pain than those in the flurbiprofen ($p = 0.030$), and control groups ($p = 0.001$) respectively. Also, HA and flurbiprofen administration decreased the pain levels comparing the control group ($p < 0.05$). All study groups showed a statistical decrease in VAS values on the 7th day compared to the control group, respectively ($p < 0.05$). There was no difference between any group in terms of pain on the 14th and 21st

**Table 1** Demographic data and periodontal parameters. *FMPS*, full-mouth plaque score; *FMBS*, full-mouth percentage bleeding score Data were presented as mean ± SD, percentage. $p < 0.05$; the significant difference between groups

| | Control | HA | HOCl | Flurbiprofen | *p* value |
|---|---|---|---|---|---|
| Women | 9(60%) | 9(60%) | 8(53.3%) | 8(53.3%) | 0.965 |
| Man | 4(40%) | 6(40%) | 7(46.7%) | 7(46.7%) | |
| Age | 37.20 ± 8.49 | 36.93 ± 9.04 | 36.33 ± 8.83 | 38.73 ± 9.76 | 0.886 |
| Number of teeth | 26.80 ± 1.47 | 26.60 ± 1.72 | 25.80 ± 1.78 | 26.13 ± 1.45 | 0.336 |
| FMPS (%) | 13.30 ± 2.21 | 13.09 ± 2.15 | 12.99 ± 1.92 | 12.55 ± 2.04 | 0.928 |
| FMBS (%) | 8.63 ± 1.47 | 8.65 ± 1.38 | 8.83 ± 1.56 | 8.68 ± 1.17 | 0.992 |

**Table 2** WHI, CM, $H_2O_2$ bubbling, and analgesic consumption of the groups. Data were presented as mean ± SD. $p < 0.05$; the significant difference between groups with the different superscripts

| Parameters | Days | Control $n = 15$ | HA $n = 15$ | HOCl $n = 15$ | Flurbiprofen $n = 15$ | *p* value |
|---|---|---|---|---|---|---|
| WHI | *3rd day* | $1.87 \pm 0.32^{a,b}$ | $2.26 \pm 0.45^{a}$ | $1.80 \pm 0.41^{b}$ | $1.86 \pm 0.35^{a,b}$ | **0.012** |
| | *7th day* | $2.66 \pm 0.48^{a}$ | $3.26 \pm 0.45^{b}$ | $2.46 \pm 0.51^{a}$ | $2.53 \pm 0.51^{a}$ | **0.001** |
| | *14th day* | $3.73 \pm 0.45^{a}$ | $4.26 \pm 0.45^{b}$ | $3.46 \pm 0.51^{a}$ | $3.47 \pm 0.52^{a}$ | **0.001** |
| | *21st day* | $4.40 \pm 0.50^{a}$ | $5.00 \pm 0^{b}$ | $4.20 \pm 0.41^{a}$ | $4.26 \pm 0.59^{a}$ | **0.001** |
| | *28th day* | 5.00 ± 0 | 5.00 ± 0 | 5.00 ± 0 | 5.00 ± 0 | 1 |
| Color match (CM) | *3rd day* | $4.33 \pm 4.87^{a,b}$ | $11.33 \pm 9.15^{a}$ | $3.33 \pm 6.17^{b}$ | $6.66 \pm 8.17^{a,b}$ | **0.022** |
| | *7th day* | $18.66 \pm 10.60^{a}$ | $40.66 \pm 10.99^{b}$ | $22.66 \pm 7.03^{a}$ | $21.33 \pm 9.15^{a}$ | **0.001** |
| | *14th day* | $59.33 \pm 7.98^{a}$ | $78.66 \pm 7.43^{b}$ | $60.66 \pm 10.99^{a}$ | $58.00 \pm 6.76^{a}$ | **0.001** |
| | *21st day* | $83.33 \pm 9.75^{a}$ | $95.33 \pm 5.16^{b}$ | $86.66 \pm 8.16^{a,b}$ | $80.66 \pm 8.83^{a}$ | **0.001** |
| | *28th day* | $96.00 \pm 5.07^{a,b}$ | $100 \pm 0^{a}$ | $96.66 \pm 4.87^{a,b}$ | $95.33 \pm 5.16^{b}$ | **0.029** |
| $H_2O_2$ bubbling | *3rd day* | 96.00 ± 6.32 | 96.00 ± 6.32 | 98.00 ± 4.14 | 97.33 ± 5.93 | 0.711 |
| | *7th day* | $80.66 \pm 8.83^{a}$ | $69.33 \pm 10.32^{b}$ | $81.33 \pm 7.43^{a}$ | $81.33 \pm 8.33^{a}$ | **0.004** |
| | *14th day* | $12.00 \pm 9.41^{a,b}$ | $8.00 \pm 9.41^{a}$ | $21.33 \pm 11.87^{b}$ | $18.66 \pm 12.45^{a,b}$ | **0.005** |
| | *21st day* | 3.33 ± 4.87 | 0 | 5.33 ± 5.16 | 4.66 ± 5.16 | 0.011 |
| | *28th day* | 0 | 0 | 0 | 0 | 1 |
| Analgesic consumption | *1st day* | $2.26 \pm 0.70^{a}$ | $1.06 \pm 0.88^{b}$ | $1.20 \pm 0.77^{b}$ | $1.66 \pm 0.72^{b}$ | **0.001** |
| | *3rd day* | $2.20 \pm 0.67^{a}$ | $1.10 \pm 0.70^{b}$ | $0.66 \pm 0.61^{b}$ | $1.33 \pm 0.97^{b}$ | **0.001** |
| | 7th day | 0.33 ± 0.61 | 0.13 ± 0.35 | 0 | 0.35 ± 0.62 | 0.156 |

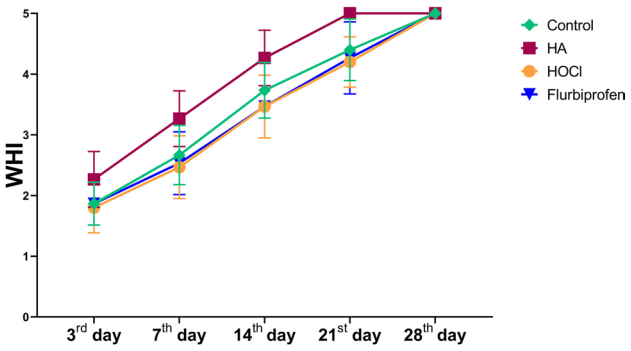


**Fig. 2** Mean WHI values of groups

days. The chewing efficiency of the patients in the HOCl group was found to be significantly better in the flurbiprofen ($p=0.001$) and control groups ($p=0.016$) on the 1st day, and in the control group ($p=0.033$) on the 3rd day. HOCl reduced the burning sensation compared to the flurbiprofen group ($p=0.004$) and the control group ($p=0.039$) on the 1st day. The burning sensation on the 3rd and 7th day in all study groups was found to be significantly lower than the control group, respectively ($p<0.05$) (Table 3, Fig. 3).

HA group showed better CM with contiguous palatal tissue compared to the HOCl group ($p=0.037$) on the 3rd day and those of all groups on the 7th and 14th days ($p<0.05$). On day 21, the HA group had better results than the control ($p=0.003$) and flurbiprofen ($p=0.001$) groups, but on day 28, it achieved a better color match than flurbiprofen ($p=0.034$) alone (Table 2, Fig. 4).

On the 7th day, the HA group showed less $H_2O_2$ bubbling than the control ($p=0.030$), HOCl ($p=0.015$), and flurbiprofen groups ($p=0.015$).On the 14th and 21st days, HA showed significant differences only with HOCl and flurbiprofen ($p<0.05$). On the 28th day, epithelialization was completed in all groups (Table 2).

The photographs of palatal area were analyzed, and the CE was assessed visually. The HA group showed

**Table 3** VAS pain, chewing, and burning values of groups. Data were presented as min–max (median). $p<0.05$; the significant difference between groups with the different superscripts

| Parameters | Days | Control $n=15$ | HA $n=15$ | HOCl $n=15$ | Flurbiprofen $n=15$ | $p$ value |
|---|---|---|---|---|---|---|
| VAS pain | *1st day* | 3–8(5)$^{a}$ | 2–8(5)$^{a,b}$ | 0–6(3)$^{b}$ | 1–7(5)$^{a}$ | **0.005** |
| | *3rd day* | 5–8(7)$^{a}$ | 1–5(3)$^{b,c,d}$ | 0–3(2)$^{c}$ | 1–6(3)$^{d}$ | **0.001** |
| | *7th day* | 3–6(5)$^{a}$ | 0–3(1)$^{b}$ | 0–1(0)$^{b}$ | 0–4(1)$^{b}$ | **0.001** |
| | *14th day* | 0 | 0 | 0 | 0 | 1 |
| | *21st day* | 0 | 0 | 0 | 0 | 1 |
| VAS chewing | *1st day* | 3–7(5)$^{a}$ | 2–7(4)$^{a,b}$ | 0–7(2)$^{b}$ | 3–7(5)$^{a}$ | **0.001** |
| | *3rd day* | 2–7(4)$^{a}$ | 0–5(3)$^{a,b}$ | 0–4(2)$^{b}$ | 1–6(3)$^{a,b}$ | **0.021** |
| | *7th day* | 0–2(1) | 0–3(1) | 0–2(0) | 0–2(1) | 0.134 |
| | *14th day* | 0–2(0) | 0 | 0 | 0 | 0.107 |
| | *21st day* | 0 | 0 | 0 | 0 | 1 |
| VAS burning | *1st day* | 2–6(4)$^{a}$ | 0–6(4)$^{a,b}$ | 0–4(2)$^{b}$ | 1–8(4)$^{a}$ | **0.004** |
| | *3rd day* | 3–8(6)$^{a}$ | 0–4(2)$^{b}$ | 0–3(1)$^{b}$ | 1–7(3)$^{b}$ | **0.001** |
| | *7th day* | 4–8(6)$^{a}$ | 0–3(0)$^{b}$ | 0$^{b}$ | 0–5(0)$^{b}$ | **0.001** |
| | *14th day* | 0–1(0) | 0 | 0 | 0–2(0) | 0.106 |
| | *21st day* | 0 | 0 | 0 | 0 | 1 |

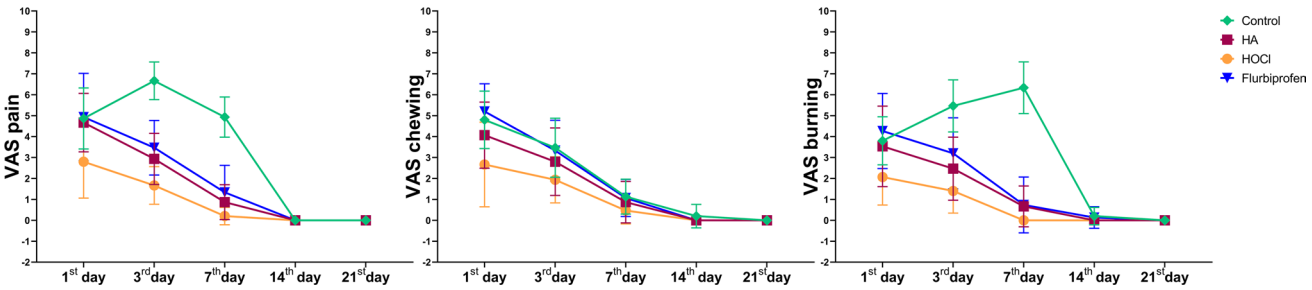


**Fig. 3** Mean VAS pain, chewing, and burning values of groups

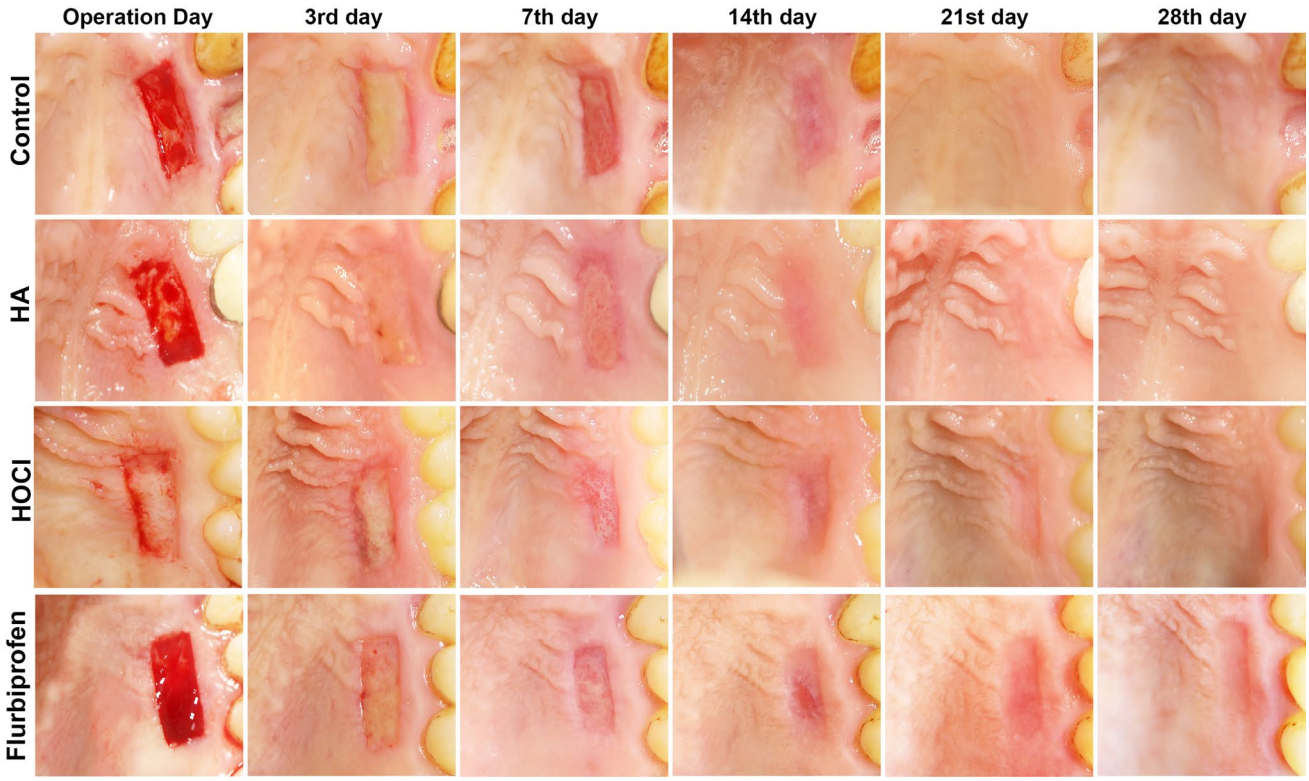


**Fig. 4** In situ images of groups on the operation, 3rd, 7th, 14th, 21st, and 28th days

**Table 4** Complete epithelization (CE) and delayed bleeding (DB) values of the groups. Data were presented as count (%of total). $p < 0.05$; the significant difference between groups with the different superscripts

| Parameters | Days | | Control $n=15$ | HA $n=15$ | HOCl $n=15$ | Flurbiprofen $n=15$ | $p$ value |
|---|---|---|---|---|---|---|---|
| Complete epithelization (CE) | *3rd day* | No | 15(100%) | 15(100%) | 15(100%) | 15(100%) | |
| | *7th day* | No | 15(100%) | 15(100%) | 15(100%) | 15(100%) | |
| | *14th day* | No | 11(73.3%) | 7(46.7%) | 12(80%) | 11(73.3%) | 0.054 |
| | | Yes | 4(26.7%) | 8(53.3%) | 3(20%) | 4(26.7%) | |
| | *21th day* | No | 5(33.3%)$^{a,b}$ | 0(0)$^{a}$ | 8(53.3%)$^{b}$ | 7(46.7%)$^{b}$ | **0.010** |
| | | Yes | 10(66.7%)$^{a,b}$ | 15(100%)$^{a}$ | 7(46.7%)$^{b}$ | 8(53.3%)$^{b}$ | |
| | *28th day* | Yes | 15(100%) | 15(100%) | 15(100%) | 15(100%) | |
| Delayed bleeding (DB) | *1st day* | No | 8(53.3%) | 12(80%) | 7(46.7%) | 11(73.3%) | 0.181 |
| | | Yes | 7(46.7%) | 3(20%) | 8(53.3%) | 4(26.7%) | |
| | *3rd day* | No | 10(66.7%) | 14(93.3%) | 10(66.7%) | 12(80%) | 0.200 |
| | | Yes | 5(33.3%) | 1(6.7%) | 5(33.3%) | 3(20%) | |
| | *7th day* | No | 12(80%) | 15(100%) | 13(86.7%) | 13(86.7%) | 0.199 |
| | | Yes | 3(20%) | 0(0) | 2(13.3%) | 2(13.3%) | |

significantly better epithelialization than the HOCL and flurbiprofen groups on the 21st day (Fig. 4, Table 4). There was no difference between the groups in terms of delayed bleeding on the day of the 1st, 3rd, and 7th days postoperatively (Table 4, Fig. 4).

## Discussion

The most unfavorable condition after FGG surgery for patients is palatal donor site morbidity. Numerous clinical studies have focused on enhancing palatal wound healing

and reducing patient discomfort [8, 26, 32, 33]. However, there is no study in the literature evaluating the effects of HOCl on palatal wound healing after periodontal plastic surgery. The present randomized clinical study was designed to investigate the therapeutic effects of different topical agents on the secondary wound healing of the donor palatal region and the patient's discomfort after the FGG procedure. The primary study outcome (WHI) indicated that the HA group revealed the best healing scores up to 21 days postoperatively. Concomitantly, higher rates of CE on the 14$^{th}$ day were observed in the HA group compared to the other study groups. However, HOCL resulted in lower VAS levels for the pain, chewing, and burning sensation parameters compared to the controls.

Many steps are necessary for wound healing to take place, among them; cell differentiation, proliferation, migration, and collagen deposition [34, 35]. HA is a member of GAGs, which is the main component of the extracellular matrix (ECM) and has beneficial effects on wound healing by reducing inflammation, increasing vascularization, and collagen synthesis [36–38]. HA is involved in all steps of the wound-healing process [39]. Yıldırım et al. [26] compared two different high molecular weight HA gels (0.2% and 0.8%) in terms of donor site healing. According to the results, healing was found to be better in the 0.2% gel group on the 14$^{th}$ day, while both HA groups showed better epithelialization on the 21$^{st}$ day than the control. Chen et al. [40] reported that high molecular weight HA when combined with povidone-iodine, significantly improved wound healing, and promoted both cell proliferation and neovascularization at the wound site compared to low molecular weight HA. Increased fibroblast proliferation, rapid wound closure, and increased inflammatory cell infiltration have been reported after the topical application of HA [41]. In addition, in an animal study, it was found that the use of HA in the wound healing model in groups treated with HA increased the levels of fibroblast, collagen I, and collagen III consequently accelerating wound healing [42]. There are studies indicating that HA stimulates keratinocyte migration and proliferation and has a positive effect on re-epithelialization [43]. Since HA plays a curative role in every step of wound healing, we also obtained the best wound healing scores in the groups treated with HA in our study, and complete epithelialization was achieved in the HA group in 21 days compared to the other groups.

In our study, VAS scores on the 3$^{rd}$ and 7$^{th}$ days were found to be lower in the HA group compared to the control group, and analgesic consumption of patients was lower in the HA group on the 1$^{st}$ and 7$^{th}$ days compared to the control group. After FGG surgery, locally applied HA gel (a mixture of cross-linked (1,6%) and natural (0,2%) HA) was compared with the control group and followed for six months. According to the results of the study, there was no difference between the two groups in terms of CM, and pain levels were higher in the control group compared to the HA group in the first seven days [44]. Hassan et al. [45] compared 0.2% HA gel, MEBO, and the control group, and it was found that the study groups showed significantly lower VAS scores on the 2$^{nd}$ and 3$^{rd}$ days compared to the control group. They stated that from the 4th day, the patients did not have any pain although there was no difference between the groups in terms of wound size at any time. These differences may be due to differences in HA molecular weight and concentration. In our study, we used high molecular weight HA at 0.6% concentration. High molecular weight HA exhibits anti-inflammatory and immunosuppressive properties. It has been reported that high molecular weight HA inhibited the IL-1β expression in a rabbit osteoarthritis study [46]. In an osteoarthritis model, high molecular weight HA downregulated IL-8 and the inducible nitrous oxide synthase gene expression and downregulated TNF-α gene expression in IL-1-stimulated fibroblast-like synoviocytes [47]. Campo et al. [48] found a reduction of mRNA expression and protein production for TNF-α, IL-1β, IL-17, matrix metalloproteinases-13, and the inducible nitrous oxide synthase gene in high molecular weight HA-treated arthritic mice. Furthermore high molecular weight HA was found to decrease the IL-1β, IL-6, TNF-α, and nitric oxide in microglial cells exposed to lipopolysaccharide [49]. Considering the necessity of increasing certain cytokines for the formation of acute pain, HA could decrease the perception of pain in patients by these mechanisms.

As the risks of infectious diseases increase around the world, the need for an effective broad-spectrum antimicrobial agent is increasingly becoming a therapeutic imperative. Because it inactivates the SARS-CoV-2 virus, HOCl has become popular in dentistry as a disinfectant and antiseptic during the COVID-19 pandemic [50, 51]. Naturally, respiratory bursts during the activation of neutrophils produce peroxide ($H_2O_2$) and the activated granule enzyme myeloperoxidase converts $H_2O_2$ to HOCl in the presence of $Cl^-$ and $H^+$ [52]. The resulting HOCl contributes to the bactericidal activity of neutrophils and is thought to cause tissue damage in areas of inflammation. In many studies, it has been stated that the use of HOCl has positive effects on wound healing [53]. In an in-vitro study, the effectiveness of NaClO/HClO solutions on wound healing was investigated and an increased antimicrobial effect was associated with decreased viability of keratinocytes and fibroblasts. The authors noted that the microbicidal effects almost always have a certain negative impact on cell proliferation and viability [54]. HOCl is a weak acid and an oxidant and also has high interaction potential with other molecules in a redox reaction, resulting in the formation of reactive oxygen species (ROS: hydrogen peroxide, superoxide, hydroxyl radicals, oxygen) [54, 55]. Excessive ROS may alter and/or degrade ECM proteins, resulting in impaired dermal

fibroblast and keratinocyte function [56]. Considering the results of our study, lower WHI scores were obtained in the HOCl-administered group compared to the other groups, including the controls, in all time periods. Studies have also emphasized that HOCl is a selective oxidant that can easily react with cellular proteins like fibronectin, thrombospondin, and laminin, and may also cause extracellular matrix fragmentation and tissue denaturation [57, 58]. The resulting protein damage was found to be related to the amount of HOCl administered rather than the concentration [54, 58]. This result can be explained as follows: HOCl application causes an increased ROS in the palatal region and may result in a delay in wound healing. When HOCl and saline were compared in a histological study, although the number of methicillin-resistant Staphylococcus aureus (MRSA) was found to be decreased in the HOCl group compared to the control, there was no difference between the two groups in terms of epithelial thickness and granulation tissue formation [59]. In a randomized controlled trial (RCT) performed on the acute wound model, the use of HOCl was beneficial for epithelialization in the first days, but no difference was found in terms of re-epithelialization versus saline use on day 10. In addition, there was no significant difference between the two groups in VAS pain scores [60]. Mekkawy et al. [61] investigated the efficacy of HOCl on septic traumatic wounds in an RCT and found lower VAS scores up to 14 days than the control group, consistent with our study. In our study, pain, chewing, and burning scores evaluated by VAS in the HOCl group were found to be significantly lower than the control group up to 7 days. The side effect of a bacterial infection can be a tender, painful wound [62]. Proinflammatory cytokines such as ILs, TNF-α, granulocyte–macrophage colony-stimulating factor (GM-CSF), and monocyte chemoattractant protein-1 may be potential biomarkers for acute surgical wound pain [63]. HOCl has anti-inflammatory and immunomodulatory properties and reduces histamine, leukotriene B4, IL-6, and IL-2 activities [64]. Some authors have suggested that super-oxidized solutions act as a mast cell membrane stabilizing inhibitor, and diminish the mast-cell degranulation induced by IgE-antigen receptor cross-linking [65]. The fact that the VAS scores in our study were lower in the HOCl-administered groups than in the other groups may be the result of the suppression of inflammation due to the effects of HOCl, an antibacterial agent, on cytokine expression.

Flurbiprofen decreases prostaglandin synthesis by inhibiting the COX-2 enzyme induced immediately in response to injury [66] but prostaglandins organize the cellular proliferation, vascular permeability, and angiogenesis, involved in wound healing [67]. In an animal model, COX-2 inhibitors caused delayed re-epithelialization in excisional wounds at cutaneous tissue [68]. Additionally, it was reported that COX-2 selective inhibitors resulted in significant inhibition of angiogenesis [69]. In wounded gastric epithelial cells, Pai et al. indicated that NSAIDs decrease both basal and epidermal growth factor induced re-epithelialization [70]. In our study, mean WHI values in the flurbiprofen group were similar for the control and HOCL groups at all-time points, but significantly lower than the HA group. Although the prevalence of CE was lower on the 21$^{st}$ day compared to the control group, it was not statistically significant. Isler et al. investigated the effects of topical flurbiprofen on the palatal donor site after both subgingival connective tissue graft (SCGT) and FGG procedures. According to their results, flurbiprofen resulted in delayed epithelization on the 21$^{st}$ day compared to the placebo group, and epithelization was completed within 42 days for all patients [33]. Contrary to these results, the prevalence of CE was 100% in 28 days in our study. It was shown that residual tissue thickness at the donor site after FGG harvesting affects the speed of palatal wound filling directly [71]. The lower graft thickness obtained in the present study may have resulted in faster re-epithelization in the palatal donor area. Many studies show that topically administered NSAIDs are effective in the management of postoperative pain after tonsillectomy [10, 72]. Koray et al. found similar VAS pain scores of NSAI spray (benzydamine hydrochloride) application compared to hyaluronic acid spray (0.2%) after third molar surgery [73]. In the study of Isler et.al, flurbiprofen spray decreased the VAS pain levels compared to the placebo group throughout the study period [33]. Consistent with the results of these studies, in the present study, VAS scores in the flurbiprofen group were similar HA group but were lower than controls on the 3$^{rd}$ and 7$^{th}$ days postoperatively.

The present study had some limitations. VAS provides a subjective evaluation of the pain, chewing, and burning sensation rather than an objective parameter. Although the VAS scores showed a statistically significant difference between the groups at the time of evaluation, there was no difference between the groups in terms of analgesic consumption by the patients. Despite the statistically significant differences detected between groups, they may only be of very low clinical relevance. Also, using a locally applied therapeutic agent by patients may have caused them to report lower VAS scores in study groups compared to the controls. Another limitation of our study is the inability to examine cytokine expression by histological examination due to ethical barriers.

## Conclusion

Clinicians should consider possible beneficial effects on the secondary wound healing process and patients' discomfort; high molecular weight HA may be the first choice for the management of palatal donor site morbidity after FGG

procedures. Although the use of HOCl and flurbiprofen reduced the pain compared to the control group, delayed epithelialization was observed due to possible effects on the inflammatory phase of wound healing.

**Author contribution** Both authors have contributed equally to the work.

**Data Availability** The data that support the findings of this study are available from the corresponding author, upon reasonable request.

## Declarations

**Competing interests** The authors declare no competing interests.

**Ethics approval** Permission was obtained from the Ethics Committee of Pamukkale University (05.03.2019/05) for the study protocol.

**Conflict of interest** The authors declare no conflict of interest.

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